Docket No: C38435/109700CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :)

Akira ASAKURA *et al.*) Examiner: M. Walicka

Serial No.: 09/470,667) Art Unit: 1652

Filed: December 22, 1999)

For: **NOVEL ALCOHOL/ALDEHYDE DEHYDROGENASES**)

#18
B.9.1
12/30/02

Commissioner for Patents
Washington, D.C. 20231

FIRST DECLARATION OF DR. MASAKO SHINJOH UNDER 37 C.F.R. § 1.132

Sir:

I, Masako Shinjoh, a citizen and resident of Japan, hereby declare as follows:

1. I am employed by Nippon Roche Research Center of Nippon Roche K.K., Kajiwara 200, Kamakura-shi, Kanagawa-ken 247-8530, Japan (hereafter "NRKK"). I currently hold the position of genetic engineer at NRKK. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. I am a coinventor of U.S. patent application No. 09/470,667 (the '667 application). The present application discloses and claims, in part, recombinant polypeptides that have alcohol/aldehyde

dehydrogenase activity, recombinant expression vectors containing DNA sequences that encode such polypeptides, recombinant organisms containing such expression vectors, processes for producing recombinant enzymes having alcohol/aldehyde dehydrogenase activity, and processes for producing, *e.g.*, 2-keto-L-gulonic acid (2-KGA).

3. As set forth in more detail in the SECOND DECLARATION OF DR. MASAKO SHINJOH UNDER 37 CFR §1.132 filed concurrently herewith, after reviewing the Sequence Listing filed with the '667 application, how the nucleotide and amino acid sequences that make up the Sequence Listing were incorporated into the '667 application, and the original nucleotide printouts from the sequencing machine used to read the experimentally derived nucleotide sequences, I have come to the conclusion that SEQ ID NOs:1, 3, and 7 each contain a single base (SEQ ID NOs:1 and 3) or a single amino acid (SEQ ID NO:7) error that arose through typing errors.

4. By way of background, SEQ ID NOs: 1 and 3, as disclosed in the '667 application, were derived from chromosomal DNA obtained from a cell culture of *Gluconobacter oxydans* strain DSM No. 4025. SEQ ID NOs: 1 and 3 encode subunits (Enzyme A and Enzyme A",

respectively) of an alcohol/aldehyde dehydrogenase ("AADH"). AADH is used to produce, *e.g.*, 2-keto-L-gluconic acid (2-KGA), an intermediate in the production of L-ascorbic acid (vitamin C). SEQ ID NO:7 is the amino acid sequence deduced from the nucleotide sequence of SEQ ID NO:3.

5. To confirm my belief that typographical errors were introduced into SEQ ID NOs:1, 3, and 7 of the '667 application, and that such errors would have been readily determined by one skilled in this art because of the public availability of the starting material (*i.e.*, the same cell line used to generate the nucleotide sequence from which SEQ ID NOs:1 and 3 were determined in the '667 application), I have supervised and coordinated NRKK's attempt to confirm the errors in the nucleic acid sequences of SEQ ID NOs:1 and 3, and the amino acid sequence deduced from SEQ ID NO:3 and set forth as SEQ ID NO:7 in the '667 application.

6. On June 6, 2000, at my direction Ms. Ayano Makino-Matsubara, who works in the NRKK Export/Import & Purchasing department, sent an order letter, via facsimile, to the International Depository Authority, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ"), a publicly available cell depository, requesting a sample of *Gluconobacter oxydans* strain DSM 4025,

the same strain used to clone and sequence the polynucleotide sequences identified in the '667 application as SEQ ID NOs:1 and 3. A copy of the order letter to the DSMZ is attached as Exhibit 2. Accompanying the order letter was a purchase request from NRKK (attached as Exhibit 3) and Form DSMZ-BP/13 (a REQUEST FOR FURNISHING SAMPLES OF DEPOSITED MICROORGANISM), which is attached as Exhibit 4. On the same day, Ms. Makino-Matsubara also sent to DSMZ, by common mail carrier, hard copies of the order letter, purchase order, and Form DSMZ-BP/13 transmitted via facsimile earlier in the day. (*See* Exhibit 5).

7. On June 19, 2000, I received a package from DSMZ postmarked June 14, 2000. The package contained an Invoice (No. 2002963 (Exhibit 6)), an ampoule labeled as containing lyophilized cells of DSM 4025, a delivery slip (no. 2002963 (Exhibit 7)), directions for cultivating the reconstituted DSM 4025 cells (Exhibit 8), and a copy of a RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT, Form DSM-BP/4 (Exhibit 9). I also took photographs of the contents of the package. Two of these photographs are enclosed as Exhibits 10 and 11.

8. On the same day I received the labeled ampoule containing the lyophilized DSM 4025 cells, I placed the ampoule in a refrigerator in

Building 27, room 524 of NRRC for storage until I could finalize arrangements for the cloning and sequencing of chromosomal DNA derived from these cells. This refrigerator is accessible only by authorized personnel of NRKK.

9. On August 11, 2000, I also took photographs of the refrigerator containing the labeled ampoule. Three representative photographs are attached as Exhibits 12, 13, and 14. I also sent an e-mail to Mr. Mashita on August 11, 2000 to confirm that the DSM 4025 cells would be forwarded to Sawady on August 16, 2000 (a copy of the original email in Japanese is enclosed as Exhibit 15 and its translation in English as Exhibit 16).

10. On August 16, 2000, I retrieved the labeled ampoule containing the lyophilized DSM 4025 cells from the refrigerator in Building 27. I then packaged the ampoule for transport, and deposited it with a local delivery service (Takkyubin) for overnight delivery to Mr. Masao Mashita, sales director of K. K. Sawady Technology, 1-29-10, Marno-cho, Itabashi-ku, Tokyo, 174-0063, Japan ("Sawady"). A copy of the Takkyubin delivery slip and a certified English translation of the relevant part thereof are attached as Exhibits 17 and 18, respectively.

11. Along with the ampoule, the package I forwarded to Mr. Mashita contained a letter providing sequence information for generating forward and reverse primers to be used by Sawady in the cloning and sequencing of the relevant parts of SEQ ID NOs:1 and 3.

12. In sum, the ampoule containing the lyophilized DSM 4025 cells that I received from DSMZ on June 19, 2000 was the same ampoule that I forwarded to Mr. Mashita at Sawady on August 16, 2000.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: September 18, 2002

Masako Shinjoh

Masako Shinjoh

CURRICULUM VITAE of Masako Shinjoh As of August 28, 2002

Scientist
Department of Applied Microbiology
Nippon Roche K.K.
Nippon Roche Research Center
200 Kajiwara Kamakura, Japan
247-8530.

Phone: +81-467-47-2226
FAX: +81-467-45-6812
E-mail: masako.shinjoh@roche.com

Education & Research Experience:

1a. Scientist (April 1979 to date) at Dept of Applied Microbiology, Nippon Roche K.K., Nippon Roche Research Center at Kamakura, Japan, which belongs to Vitamin and Fine Chemical Division in Hoffmann-La Roche

This work includes improvement of microorganisms producing vitamin or its precursor by conventional method and genetic engineering.

1b. Visiting scientist (Jan. to March 1982) at Research Institute of Molecular Biology at Nutley, NJ, USA, which belonged to Hoffmann-La Roche. Objectives: to exchange scientific information and technical transfer of genetic engineering skills.

2. Ph.D. (Jan. 12, 1996)
Ph.D. in Engineering from Department of Fermentation Technology, Osaka University, Osaka, Japan.

The title of the Thesis is "Metabolic engineering for 2-keto-L-gulonic acid production in

Gluconobacter".

3. Master Degree (April 1977 to March 1979)

Master in Engineering from Department of Fermentation Technology, Osaka University, Osaka, Japan.

The projects involved were

"Characterization of bacteriophage of bacitracin-produing *Bacillus*"

"Application of plasmid on fermentation production: factors responsible for stabilization of hybrid plasmids carrying trytophane operon in *E. coli* ."

4. Bachelar Degree (April 1975 to March 1977)

Department of Fermentation Technology, Osaka University, Osaka, Japan.

The projects involved were

"In vitro synthesis of alpha-amylase of *Bacillus*"

5. Professional field

Microbiology
Fermentation technology
Genetic engineering

6. Memberships

a) The Society for Bioscience and Bioengineering
b) Japan Society for Bioscience, Biotechnology, and Agrochemistry

7. Personal information:

Female,
Japanese citizen,
Birthday: 20th February, 1955

LIST OF PUBLICATIONS

Original Papers by the Author

Shinjoh, M., Y. Setoguchi, T. Hoshino and A. Fujiwara. (1990)
L-Sorbose dissimilation in 2-keto-L-gulonic acid-producing mutant UV10 derived from *Gluconobacter melanogenus* IFO 3293. Agric. Biol. Chem. 54: 2257 - 2263.

Shinjoh, M., T. Sugisawa, S. Masuda, and T. Hoshino. (1994)
Efficient conversion of L-sorbosone to 2-keto-L-gulonic acid by *Acetobacter liquefaciens* strains. J. Ferment. Bioeng. 78: 476 - 478.

Shinjoh, M., and T. Hoshino. (1995). Development of a stable shuttle vector and a conjugative transfer system for *Gluconobacter oxydans*.J. Ferment. Bioeng. 79: 95 - 99.

Shinjoh, M., N. Tomiyama, A. Asakura, and T. Hoshino. (1995) Cloning and nucleotide sequencing of membrane-bound L-sorbosone dehydrogenase gene of *Acetobacter liquefaciens* IFO 12258 and its expression in *Gluconobacter oxydans*. Appl. Environ. Microbiol. 43: 1064 - 1069.

Shinjoh, M, M., Tazoe, and T. Hoshino. (2002) NADPH-dependent L-sorbose reductase is responsible for L-sorbose assimilation in *Gluconobacter suboxydans* IFO 3291. J. of Bacteriol., 84: 861 - 863.

Miyazaki, T., N. Tomiyama, M. Shinjoh, and T. Hoshino. (2002) Molecular cloning and functional expression of D-sorbitol dehydrogenase from *Gluconobacter suboxydans* IFO3255 which requires PQQ and hydrophobic protein SldB for the activity development in *E.coli*. (2001) Biosci. Biotechnol. Biochem. 66: 262-270. (the corresponding author)

Shinjoh, M., N. Tomiyama, T. Miyazaki, and T. Hoshino. (2002) Main polyol dehydrogenase of *Gluconobacter suboxydans* IFO 3255, membrane-bound D-sorbitol dehydrogenase, that needs product of upstream gene, *sldB*, for activity. Biosci. Biotechnol. Biochem. (in press)

Other Publications on the work done at Hoffmann-La Roche

Sugisawa, T., T. Hoshino, S. Masuda, S. Nomura, Y. Setoguchi, M. Tazoe, M. Shinjoh, S. Someha and A. Fujiwara. (1990) Microbial production of 2-keto-L-gulonic acid from L-sorbose and D-sorbitol by *Gluconobacter oxydans*. Agric. Biol. Chem. 54: 1201 - 1209.

Hoshino, T., T. Sugisawa, M. Tazoe, M. Shinjoh and A. Fujiwara. (1990) Metabolic pathway for 2-keto-L-gulonic acid formation in *Gluconobacter oxydans* IFO 3293. Agric. Biol. Chem. 54: 1211 - 1218.

Shinjoh, M., (1990) Biotechnology of acetic acid bacteria. Su no kagaku, Asakura shoten. Tokyo. 157 - 170. (in Japanese)

Other Publications on the work done at Osaka Univ.

Imanaka, T., K. Uchida, M. Tateishi (Shinjoh), and S. Aiba. (1979) Inducible bacteriophage of *Bacillus licheniformis* ATCC 10716. Virology 95: 249 - 250.

Tsunekawa, H., M. Tateishi (Shinjoh), T. Imanaka, S. Aiba. (1981) TnA-directed deletion of the trp operon from RSF2124-trp in *Escherichia coli*.

Patent publication: USP granted including "M. Shinjoh" as the inventor

(as of Aug. 28, 2002)

	PAT. NO.	Title
1	6,407,203	Cytochrome *c* and polynucleotides encoding cytochrome *c*
2	6,146,860	Manufacture of L-ascorbic acid and D-erythorbic acid
3	6,127,156	D-sorbitol dehydrogenase gene
4	6,037,147	Cytochrome *c* and polynucleotides encoding cytochrome *c*
5	5,541,108	*Gluconobacter oxydans* strains

6 5,399,496 DNA shuttle vectors for *E. coli*, *Gluconobacter*, and *Acetobacter*

7 5,352,599 Co-enzyme-independent L-sorbosone dehydrogenase of *Gluconobacter oxydans*: isolation, characterization, and cloning and autologus expression of the gene

----------END of CV----------

Memo

Declaration SM1
Ex-T 2 1/5
+4pages

Roche

To:	Dr. Vera Weihs Ms. Ina Franc	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
Copies:	Dr. Masako Shinjoh	Department AM Research Center Nippon Roche K.K.
From:	Ayano Makino-Matsubara	Export/Import & Purchasing Technical Division Nippon Roche K.K. 6-1, Shiba 2-chome, Minato-ku, Tokyo 105-8532 Japan Tel. +81 3 5443 7047 Fax +81 3 5443 7120
Date:	June 6, 2000	

Re: Our Order for DSM4025 / Official Order Sheet (Originals)

Dear Dr. Weihs and Mrs. Franc,

Attached please find the original sheets of our order sheet and your Form DSMZ-BP/13 (sole page) as you requested.

Best regards,

Ayano Makino-Matsubara

10/30

Dc SM/
Ex-2 2/5

TELECOPIER COVERSHEET

		Sender	Addressee
Company	:	Nippon Roche K.K.	DSMZ
City	:	Tokyo	Braunschweig
Surname	:	Oshida	Dr. Weihs / Ms. Franc
First Name	:	Isao	Vera / Ina
Department	:	Import / Export & Purchasing	
Building	:		
Room No.	:		
Telephone No.	:	+81 3 5443 7047	+49 531 26 16 319
Telefax No.	:	+81 3 5443 7120	+49 531 2616 444

No. of pages of the document including this page 4

Tokyo, June 6, 2000

Re: Our Order for DSM4025 / Official Order Sheet

Dear Dr. Weihs and Mrs. Franc,

Referring to e-mail from Dr. Weihs, attached please find our official purchase order sheet together with your form DSMZ-BP/13 (sole page). Original well be delivered under separate cover.

As for Shipment, would you be so kind to deliver the goods directly to...

Dr. Masako Shinjoh
Department AM
Research Center
Nippon Roche K.K.
200 Kajiwara
Kamakura-shi
Kanagawa Pref. 247-8530
Japan

../.2

11/70

BUDAPEST TREATY ON THE INTERNATIONAL

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS

FOR THE PURPOSES OF PATENT PROCEDURE

To be completed in duplicate

REQUEST

FOR THE FURNISHING OF SAMPLES

OF DEPOSITED MICROORGANISMS

pursuant to Rule 11.3 (b)

Dc SM1

Ex-2 3/5.

TO
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1B
38124 Braunschweig
Germany

THE UNDERSIGNED HEREBY REQUESTS THE FURNISHING OF A SAMPLE OF THE MICROORGANISM IDENTIFIED HEREUNDER, IN ACCORDANCE WITH RULE 11.3 (b) OF THE REGULATIONS UNDER THE BUDAPEST TREATY

I. IDENTIFICATION OF THE MICROORGANISM

Accession number of the deposit: DSM 4025

Name of the depositor[1]: Institute of Microbiology Academia Sinica, Beijing(China)
Yin, Guanglin et al.

Identification reference given by the depositor[1]

Taxonomic designation, if any, proposed by the depositor[1]:
-

II. REQUEST FOR INFORMATION

The undersigned

(X)[2] request

( )[2] does not request

an indication of the conditions which the international depositary authority employs for the cultivation and storage of the microorganism

III. REQUESTING PARTY

Name: Nippon Roche Research Center Signature[3]: Tatsuo Hoshino

Tatsuo Hoshino

Address: 200 Kajiwara, Kamakura
Kanagawa 247-8530 Japan

Date: May 30, 2000

[1] To be indicated if known to the requesting party.
[2] Mark with a cross the applicable box.
[3] Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on behalf of the legal entity should accompany the signature(s).

5/30

Form DSMZ-BP/13 (sole page)

NIPPON ROCHE K. K.

Nippon Roche Building, 6-1, Shiba 2-Chome, Minato-ku, Tokyo, 105, Japan

To : DSMZ GmbH

Order information / Sales Dept.
Mrs. Franc / Mrs. Melenk
Telephone : +49 531 2616 319
Fax : +49 531 2616 444

Terms of Payment : Within 30 Days After Date of Invoice

Incoterms :

PURCHASE ORDER

No. : 4500018356
Date : 06. 06. 2000

Purchase Dept. : Pharma Import Tokyo
Contact Person :

Telephone : +81354437047
Fax : +81 3 5443 7120
E-mail Address :

LINE	ITEM NUMBER / ITEM DESCRIPTION / COUNTRY OF ORIGIN	QUANTITY	UNIT OF MEASURE	CURRENCY / UNIT PRICE	NET VALUE	E. T. A.
00010	4025 Gluconobacter oxydans * Referring to your price confirmation of 6 June 2000 by Dr. Weihs's * e-mail. Requested by Dr. M. Shinjoh, AM, NRRC.	1	Each	DEM 180. 00 /1Each	180. 00	30. 06. 2000
00020	Handling Fee * Referring to your price confirmation of 6 June 2000 by Dr. Weihs's * e-mail. Requested by Dr. M. Shinjoh, AM, NRRC.	1	Each	DEM 40. 00 /1Each	40. 00	30. 06. 2000
	TOTAL AMOUNT OF THE ORDER			DEM	220. 00	

Dr. Shinjoh Ex-2 4/5

We are placing purchase orders with you as above.
Please return your order confirmation immediately by telefax, duly signed.

Receipt : _____

For NIPPON ROCHE K. K.

06/41

Dc sm1 EX-2 5/5

Would you please inform us of shipping details when available.

Thank you in advance for your coperation.

Best regards,

Attach: 1) Our official order sheet (order no. 4500018356) 1 page
2) Form DSMZ-BP/13 (sole page) 1 page

/amm

14/70

PURCHASE REQUEST

Sheet No. 191992

※印は固定資産のみ記入

A 新規 / B 再発注 / C 変更	前回注文No. 45000/7532	依頼部サイン欄	Requested: M. Shinjoh	Checked: ✓ / Approved: ✓

依頼No.:

予定発注先: DSMZ

納入先:

*Credit No.: T K

依頼日: DD 04 / MM 06 / YY 2000

依頼部門コード:

依頼部門名: NRRC/ATM

担当者名(内線): M. Shinjoh (2116)

*SK No. (資産区分): S K 0

*Fixed Asset Class (固定資産品目分類):

Theme No. | C/C Code | A/C Code

Description II | 希望納期 DD / MM / YY

Group/Inventory No. | 起票課コード(袋井)

品名	単価(概算)	数量	金額(概算)
Description I: -Gluconobacter oxydans DSM 4025 - Handling Fee	180 DM + 40 DM	1 1	

(合計金額¥)

備考

依頼者受領印

購買部門記入欄		Remarks	
	Received on / /		Applied
	Ordered on / /		
	Sup. Code		
	Sup. Name		Checked
	Order No. G		
	U. Price @		
	Quantity		Approved
	Amount ¥		
	Deliv. Date / /		

Declaration SM1
Ex-3

15/70

4

010009

To be completed in duplicate

BUDAPEST TREATY ON THE INTERNATIONAL

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS

FOR THE PURPOSES OF PATENT PROCEDURE

REQUEST

FOR THE FURNISHING OF SAMPLES

OF DEPOSITED MICROORGANISMS

pursuant to Rule 11.3 (b)

Declaration SM 1
Ex-4

TO
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1B
38124 Braunschweig
Germany

THE UNDERSIGNED HEREBY REQUESTS THE FURNISHING OF A SAMPLE OF THE MICROORGANISM IDENTIFIED HEREUNDER, IN ACCORDANCE WITH RULE 11.3 (b) OF THE REGULATIONS UNDER THE BUDAPEST TREATY

I. IDENTIFICATION OF THE MICROORGANISM

Accession number of the deposit: DSM 4025

Name of the depositor[1]: Institute of Microbiology Academia Sinica, Beijing(China)
Yin, Guanglin et al.

Identification reference given by the depositor[1]:

Taxonomic designation, if any, proposed by the depositor[1]:

II. REQUEST FOR INFORMATION

The undersigned

(X)[2] request

( )[2] does not request

an indication of the conditions which the international depositary authority employs for the cultivation and storage of the microorganism

III. REQUESTING PARTY

Name: Nippon Roche Research Center Signature[3]: Tatsuo Hoshino

Tatsuo Hoshino

Address: 200 Kajiwara, Kamakura
Kanagawa 247-8530 Japan

Date: May 30, 2000

[1] To be indicated if known to the requesting party.
[2] Mark with a cross the applicable box.
[3] Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on behalf of the legal entity should accompany the signature(s).

16/70

5

Roche *TELECOPIER COVERSHEET*

		Sender	Addressee
Company	:	Nippon Roche K.K.	DSMZ
City	:	Tokyo	Braunschweig
Surname	:	Oshida	Dr. Weihs / Ms. Franc
First Name	:	Isao	Vera / Ina
Department	:	Import / Export & Purchasing	
Building	:		
Room No.	:		
Telephone No.	:	+81 3 5443 7047	+49 531 26 16 319
Telefax No.	:	+81 3 5443 7120	+49 531 2616 444

No. of pages of the document including this page 4

Tokyo, June 6, 2000

Re: Our Order for DSM4025 / Official Order Sheet

Dear Dr. Weihs and Mrs. Franc,

Referring to e-mail from Dr. Weihs, attached please find our official purchase order sheet together with your form DSMZ-BP/13 (sole page). Original well be delivered under separate cover.

As for Shipment, would you be so kind to deliver the goods directly to...

Dr. Masako Shinjoh
Department AM
Research Center
Nippon Roche K.K.
200 Kajiwara
Kamakura-shi
Kanagawa Pref. 247-8530
Japan

Dec. SM1
Ex-5 (+p2~4) 1/4

../.2

17/10

Would you please inform us of shipping details when available.

Thank you in advance for your coperation.

Best regards,

Attach: 1) Our official order sheet (order no. 4500018356) 1 page
2) Form DSMZ-BP/13 (sole page) 1 page

/amm

Dec SM1
Ex-5 3/4

12/30

NIPPON ROCHE K. K.

Nippon Roche Building, 6-1, Shiba 2-Chome, Minato-ku, Tokyo, 105, Japan

To : DSMZ GmbH

Order information / Sales Dept.
Mrs. Franc / Mrs. Melenk
Telephone : +49 531 2616 319
Fax : +49 531 2616 444

Terms of Payment : Within 30 Days After Date of Invoice

Incoterms :

PURCHASE ORDER

No. : 4500018356
Date : 06. 06. 2000

Purchase Dept. : Pharma Import Tokyo
Contact Person :

Telephone : +81354437047
Fax : +81 3 5443 7120
E-mail Address :

Dec. SM1 Ex-5 3/4

LINE	ITEM NUMBER ITEM DESCRIPTION COUNTRY OF ORIGIN	QUANTITY	UNIT OF MEASURE	CURRENCY UNIT PRICE	NET VALUE	E. T. A.
00010	4025 Gluconobacter oxydans * Referring to your price confirmation of 6 June 2000 by Dr. Weihs's * e-mail. Requested by Dr. M. Shinjoh, AM, NRRC.	1	Each	DEM 180. 00 /1Each	180. 00	30. 06. 2000
00020	Handling Fee * Referring to your price confirmation of 6 June 2000 by Dr. Weihs's * e-mail. Requested by Dr. M. Shinjoh, AM, NRRC.	1	Each	DEM 40. 00 /1Each	40. 00	30. 06. 2000
	TOTAL AMOUNT OF THE ORDER			DEM	220. 00	

06/8'

We are placing purchase orders with you as above.
Please return your order confirmation immediately by telefax, duly signed.

Receipt : _____

For NIPPON ROCHE K. K.

BUDAPEST TREATY ON THE INTERNATIONAL

RECOGNITION OF THE DEPOSIT OF MICROORGANISMS

FOR THE PURPOSES OF PATENT PROCEDURE

To be completed in duplicate

Dec SM1
EX-5 4/4

REQUEST

FOR THE FURNISHING OF SAMPLES

OF DEPOSITED MICROORGANISMS

pursuant to Rule 11.3 (b)

TO
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1B
38124 Braunschweig
Germany

THE UNDERSIGNED HEREBY REQUESTS THE FURNISHING OF A SAMPLE OF THE MICROORGANISM IDENTIFIED HEREUNDER, IN ACCORDANCE WITH RULE 11.3 (b) OF THE REGULATIONS UNDER THE BUDAPEST TREATY

I. IDENTIFICATION OF THE MICROORGANISM

Accession number of the deposit: DSM 4025

Name of the depositor[1]: Institute of Microbiology Academia Sinica, Beijing(China)
Yin, Guanglin et al.

Identification reference given by the depositor[1]

Taxonomic designation, if any, proposed by the depositor[1]:

II. REQUEST FOR INFORMATION

The undersigned

(X)[2] request

( )[2] does not request

an indication of the conditions which the international depositary authority employs for the cultivation and storage of the microorganism

III. REQUESTING PARTY

Name: Nippon Roche Research Center Signature[3]: Tatsuo Hoshino

Tatsuo Hoshino

Address: 200 Kajiwara, Kamakura
Kanagawa 247-8530 Japan

Date: May 30, 2000

[1] To be indicated if known to the requesting party.
[2] Mark with a cross the applicable box.
[3] Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on behalf of the legal entity should accompany the signature(s).

20/20

Form DSMZ-BP/13 (sole page)

6

DSMZ

Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

Cust. No. 14301
DSMZ · Mascheroder Weg 1 b · D-38124 Braunschweig

Nippon Roche K.K.
Research Center
Department AM
200 Kajiwara, Kamakura-shi
Kanagawa Pref. 247-8530
Japan

Cust. No. 14301
Versandanschrift / Shipping adress

Nippon Roche K.K.
Research Center
Department AM
Dr. Masako Shinjoh
200 Kajiwara, Kamakura-shi
Kanagawa Pref. 247-8530
Japan

RECHNUNG NR.
INVOICE NO. 2002963

Datum / Date 13.06.2000

Ihre Bestellung/Your order
Ihre Anfrage/Your request 4500018356 of 6th June 2000

Pos.	Menge Quantity	DSM-Nr. DSM-No.	Bezeichnung Description	Einzelpreis Unit Price	Nettopreis Net Price
			Release of a patent strain		
1	1	4025	Gluconobacter oxydans	180.00	180.00
Customs Tariff no. 30 02 90 50					
Code: 004					
Netweight: 10 gr.					

Declaration SM 1
Ex-6

Konten /-Accounts:
Landeszentralbank Braunschweig
Konto-Nr. / Account: 27 007 326
BLZ / Bank Code 270 000 00

NORD/LB
Konto-Nr. / Account: 2 039 220
BLZ / Bank Code: 250 500 00
SWIFT NOLADE 2 H

Postbank Hannover
Konto-Nr. / Account: 1060 79-304
BLZ / Bank Code: 250 100 30

Erfüllungsort und Gerichtsstand ist Braunschweig.
The place of jurisdiction and performance is Braunschweig.

Überweisungen an / Payment to
DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
Mascheroder Weg 1b
D-38124 Braunschweig – Germany

Versand / Sales: +49(0)531 - 26 16 - 319

Kreditkarten werden nicht akzeptiert.
Credit cards cannot be accepted.

Bitte Rechnungsnummer angeben!
Please indicate the invoice number!

Zahlbar in DEM oder Euro sofort ohne Abzug.
Payable in DEM or Euro immediately after receipt of goods without further discount.

Zwischensumme / Subtotal	180.00
Bearbeitungsgebühr / Handling fee	
Zwischensumme / Subtotal	180.00
+ % MwSt. / % VAT	
Rechnungsbetrag / Invoice total DEM / EUR	180.00

21/70

VAT-Nr./No.: DE 114 815 269

DSMZ

Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

7

Cust. No. 14301
DSMZ · Mascheroder Weg 1 b · D-38124 Braunschweig

Nippon Roche K.K.
Research Center
Department AM
200 Kajiwara, Kamakura-shi
Kanagawa Pref. 247-8530
Japan

Cust. No. 14301
Versandanschrift / Shipping adress

Nippon Roche K.K.
Research Center
Department AM
Dr. Masako Shinjoh
200 Kajiwara, Kamakura-shi
Kanagawa Pref. 247-8530
Japan

LIEFERSCHEIN NR. / DELIVERY SLIP NO. 2002963

Datum / Date 13.06.2000

Ihre Bestellung/Your order
4500018356 of 6th June 2000

Pos.	Menge Quantity	DSM-Nr. DSM-No.	Bezeichnung Description
			Release of a patent strain
1	1	4025	Gluconobacter oxydans
Customs Tariff no. 30 02 90 50			
Code: 004			
Netweight: 10 gr.			

Declaration SM
Ex-7

Lieferbedingungen

Diese Lieferbedingungen gelten, soweit nicht zwischen der DSMZ und dem Besteller schriftlich anders vereinbart, für alle Lieferungen von Kulturen durch die DSMZ:

1. Die DSMZ liefert lebensfähige und authentische Kulturen. Bei Mängeln, wie beschädigten Ampullen, kontaminierten oder nicht lebensfähigen Kulturen, ist die DSMZ unverzüglich zu unterrichten. Sind Reklamationen berechtigt, werden – soweit möglich – kostenlos Ersatzkulturen geliefert. Ist dies nicht möglich, wird der Kaufpreis erstattet. Weitere Ansprüche können nicht geltend gemacht werden.
2. Die DSMZ haftet nicht für Schäden, die aus dem Bezug und dem Gebrauch gelieferter Kulturen entstehen. Insbesondere haftet die DSMZ nicht für Schäden, die durch unsachgemäße Behandlung der Kulturen entstehen. Ferner haftet die DSMZ nicht-für mittelbar oder unmittelbar aus den Kulturen hergestellte Produkte oder Folgeschäden.

Conditions of delivery

These conditions of delivery apply to the supply of cultures from the DSMZ, unless other written agreements have been entered into between the DSMZ and the customer

1. The DSMZ supplies viable and authentic cultures. Problems, such as, damaged vials, contaminated or non viable cultures should be reported immediately to the DSMZ. Should claims found to be justified, a replacement culture will be supplied whenever possible. Should this not be possible, the purchase price will be refunded. Not further claims can be accepted by the DSMZ.
2. The DSMZ is not responsible for damage or injury which may arise resulting from the purchase and use of the cultures supplied. In particular, the DSMZ is not responsible for damage or injury resulting from the improper handling of the cultures. Furthermore, the DSMZ is not responsible for damage or injury caused by products resulting directly or indirectly from the cultures, or for any subsequent costs arising.

22/30

Please use a

II. CONDITIONS FOR CULTIVATION

Medium: YEA 23

0.3% Yeast Extract
0.3% Beef Extract
0.3% Cornsteep Liquor
1.0% Peptone
0.1% KH_2PO_4
0.02% $MgSO_4 \cdot 7H_2O$
0.1% Urea
0.1% $CaCo_3$
2.0% L-Sorbose
ad 1 l H_2O deionised

pH before sterilisation:
Sterilisation: 30 min at 121° c
pH after sterilisation: 6.5
Oxygen-realtionships:
[X] aerobic
[ ] microaerophilic
[ ] obligate anaerobic
Specific gaseous requirements: None

Incubation temperature: 30° c
Incubation time: 20-40hr
Short term storage at: 5° c
Interval of transfer: 4 weeks

Declaration SM1
Ex-A

23/10

BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE
ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN
FÜR DIE ZWECKE VON PATENTVERFAHREN

9

INTERNATIONAL FORM

The Oriental Scientific Instruments
Import and Export Corporation for
Institute of Microbiology
Academia Sinica
52 San-Li-He Rd.
Beijing
People's Republic of China

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: CGMCC No. 0119	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 4025

II. SCIENTIFIC DESCRIPTION AND/OR TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(x) a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on March 17, 1987 (Date of original deposit)[1]

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DEUTSCHE SAMMLUNG VON MIKROORGANISMEN	Signature(s) of person(s) having the power to present the International Depositary Authority or of authrorized official(s): H. Hippe
Address:	Grisebachstrasse 8 D-3400 Göttingen	Date: March 25, 1987

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired, where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date ist the date on which the microorganism was received by the international depositary authority.

Form DSM-BP/4 (sole page) 1186

Declaration SM 1

Ex-9

24/30

DcSM1
Ex-10
June 13, 2000 Sa

DcSM1
Ex-11
June 13, 2000 Sa

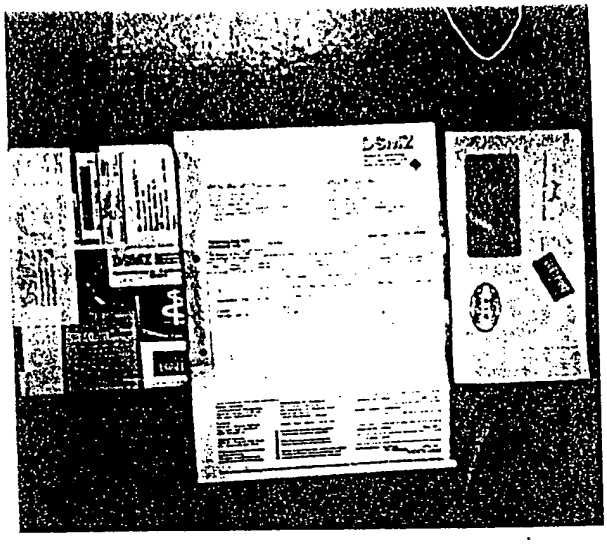
000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K
Ex-13

000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K
Ex-14

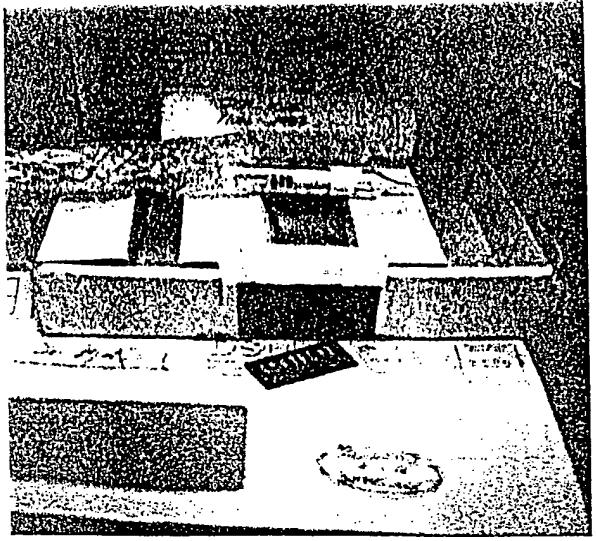
000811
DSM4025 stock in refrigerator
Bldg 27/Rm 54K
Ex-12

Declaration SM1
Ex 10 ~ 14

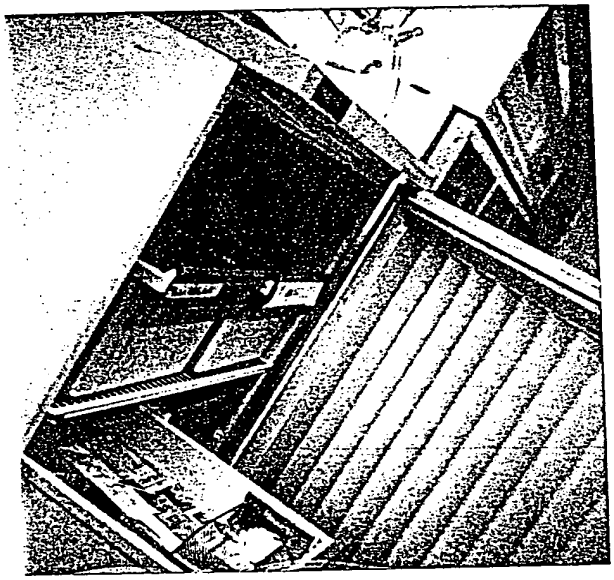
25/70



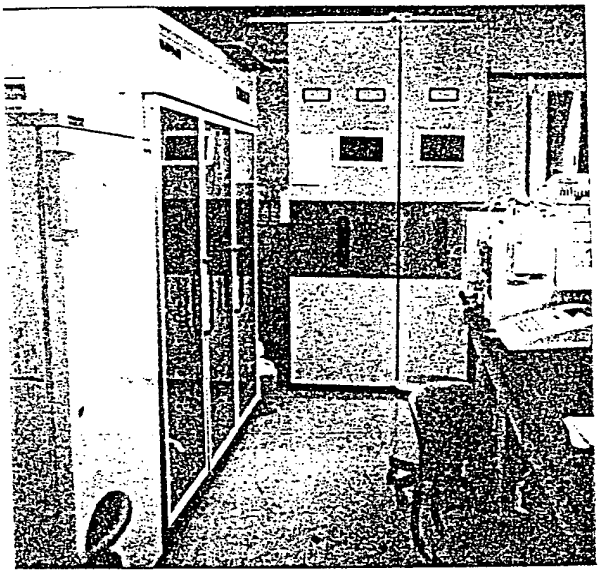
DcSM1
Ex-10
June 13, 2000 Sa



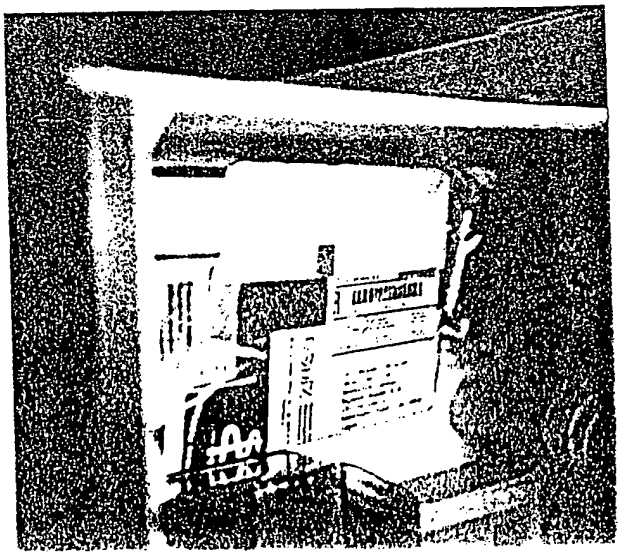
DcSM1
Ex-11
June 13, 2000 Sa



000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54X
Ex-13



000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54X
Ex-14

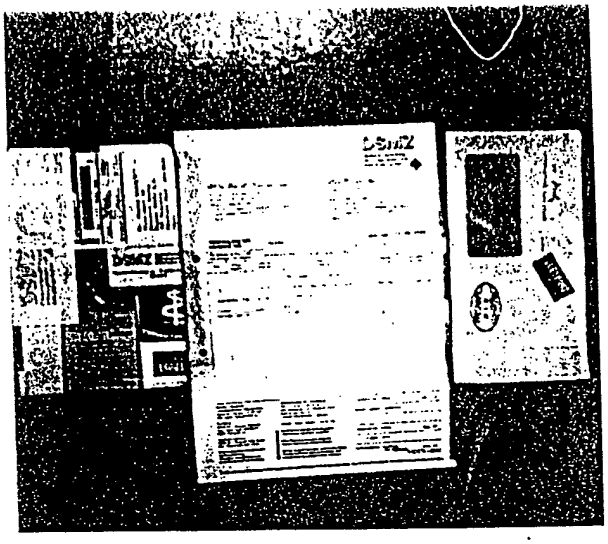


000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54X
Ex-12

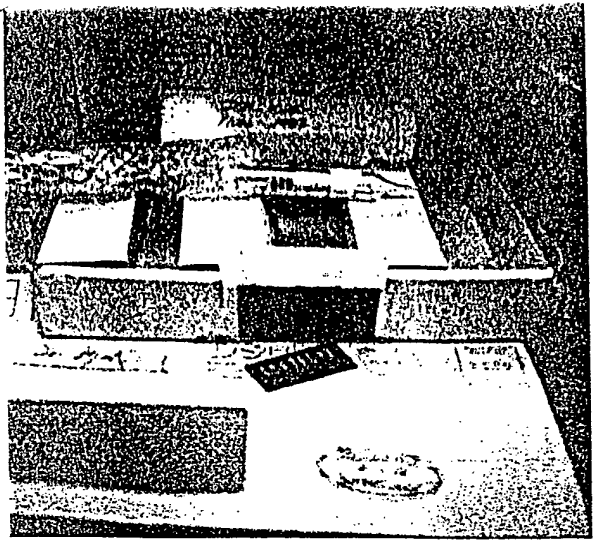
BEST AVAILABLE COPY

Declaration SM1
Ex 10 ~ 14

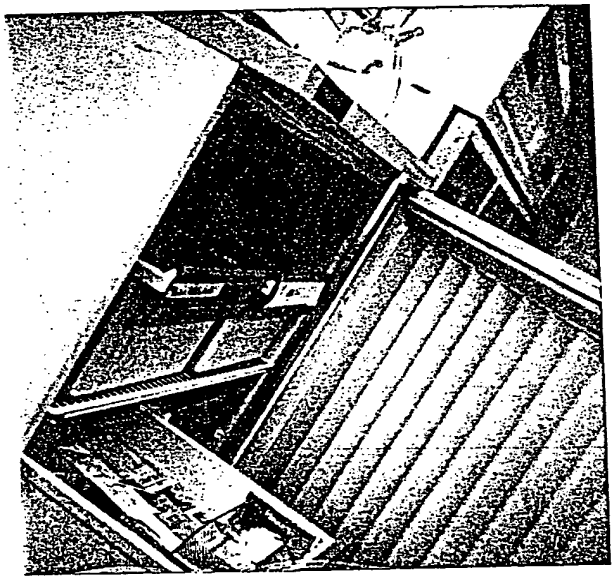
25/70



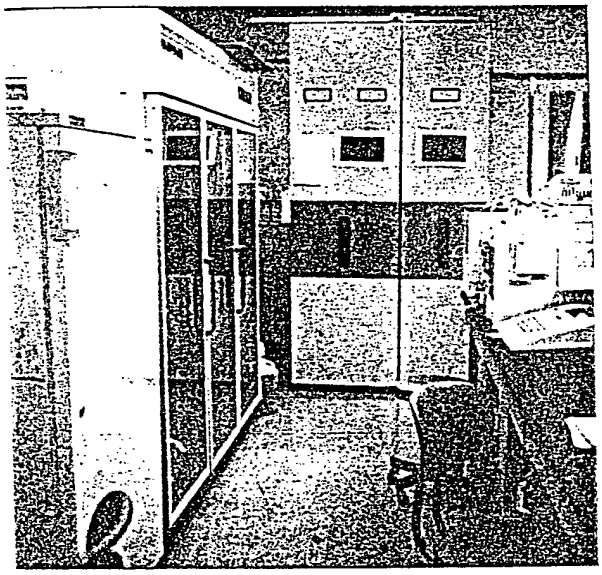
DcSM1
Ex-10
June 13, 2000 Sa



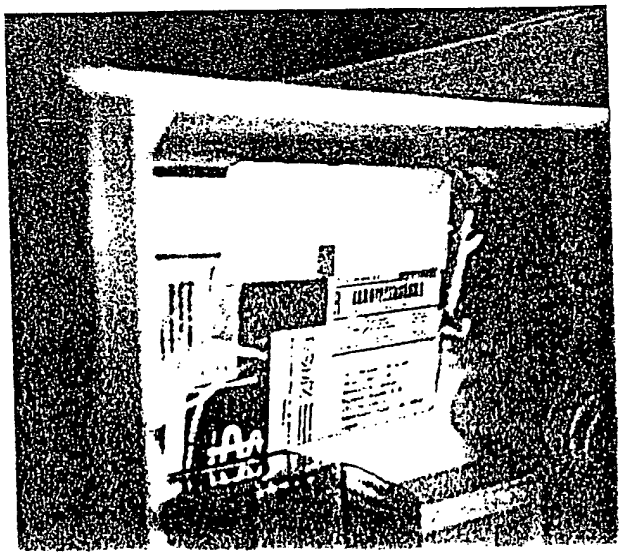
DcSM1
Ex-11
June 13, 2000 Sa



000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K
Ex-13



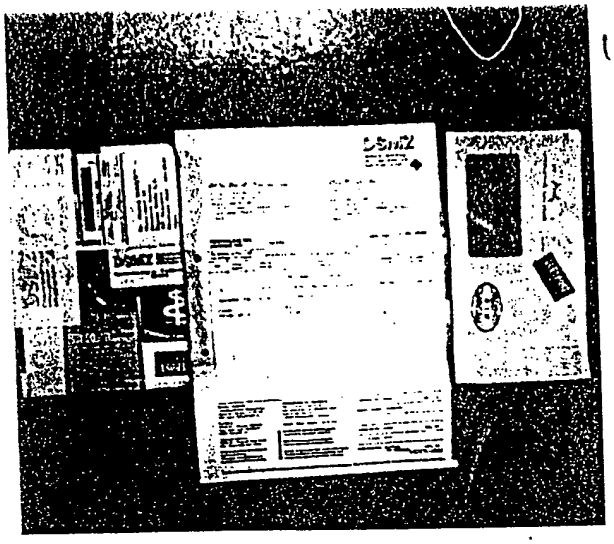
000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K
Ex-14



000811
DSM4025 stock in refrigeration
Bldg. 27/Rm 54K
Ex-12

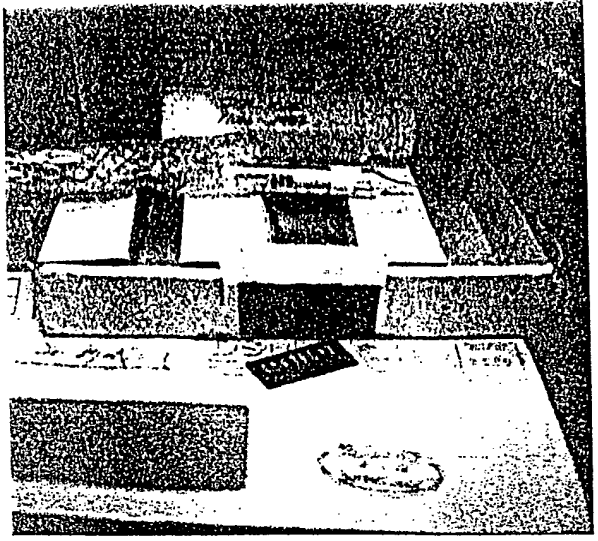
Declaration SM1
Ex 10 ~ 14

25/70



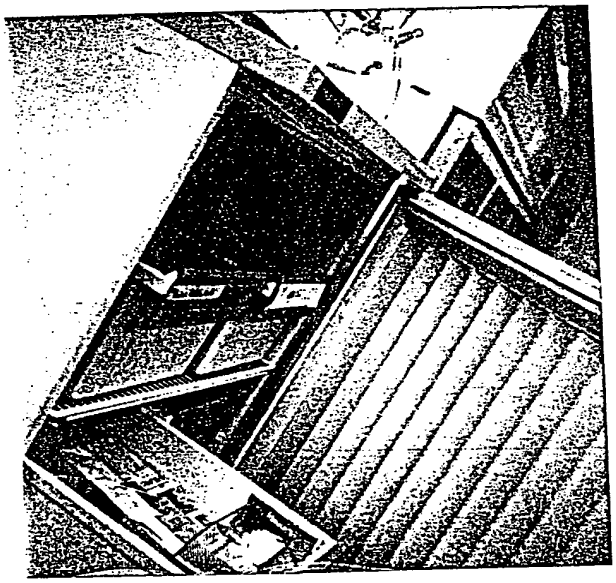
June 13, 2000 Sa

Dc SM1
Ex-10



June 13, 2000 Sa

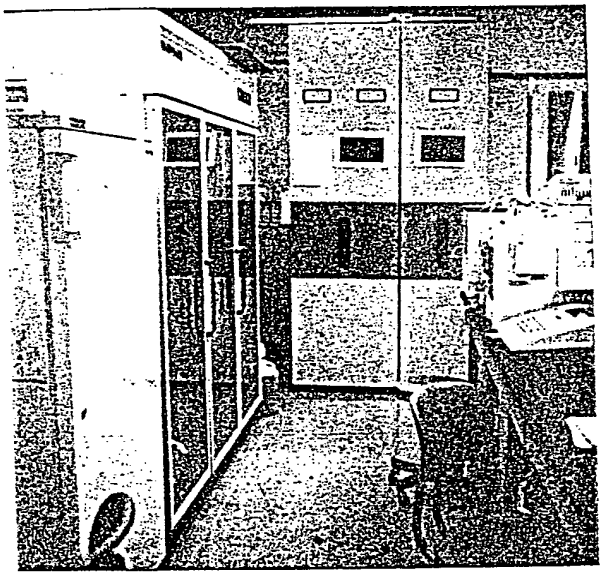
Dc SM1
Ex-11



000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K

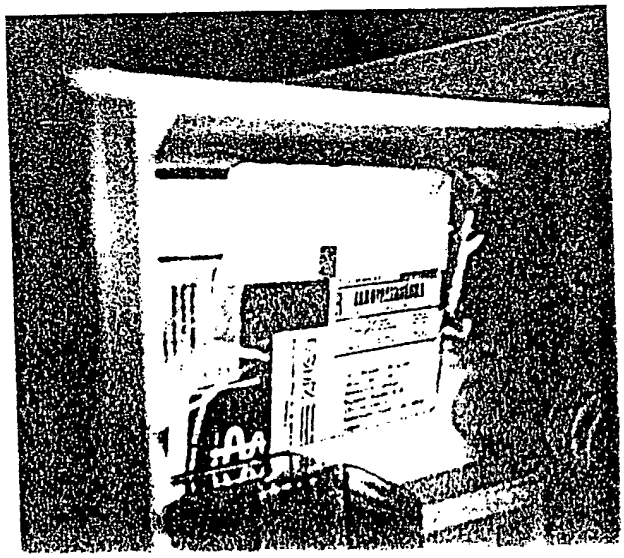
Ex-13

Ex-14 →



000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K

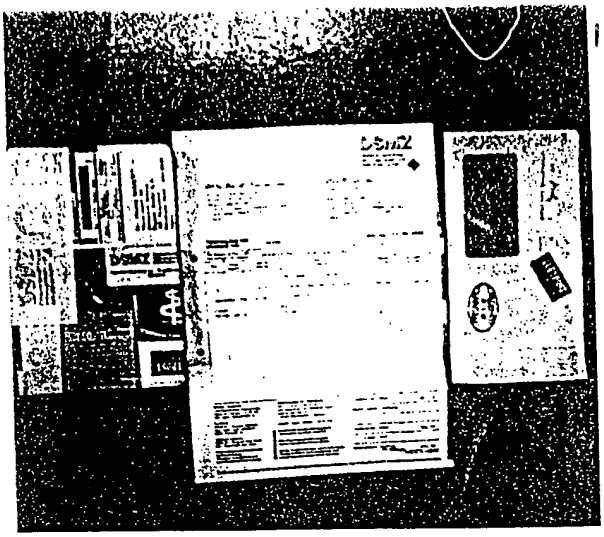
Ex-12 →



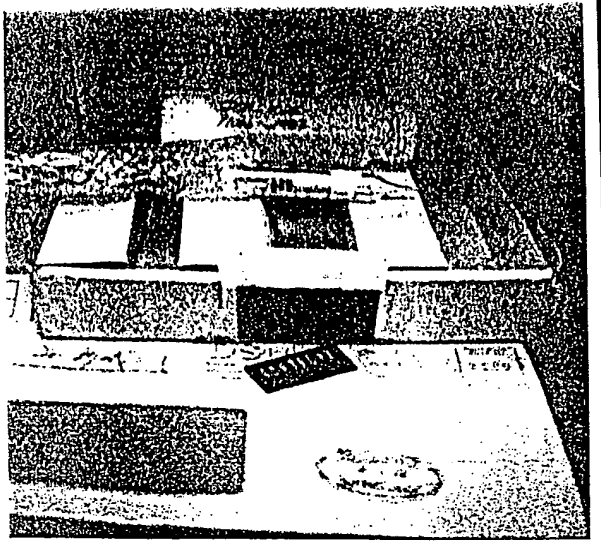
000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K

Declaration SM1
Ex 10 ~ 18

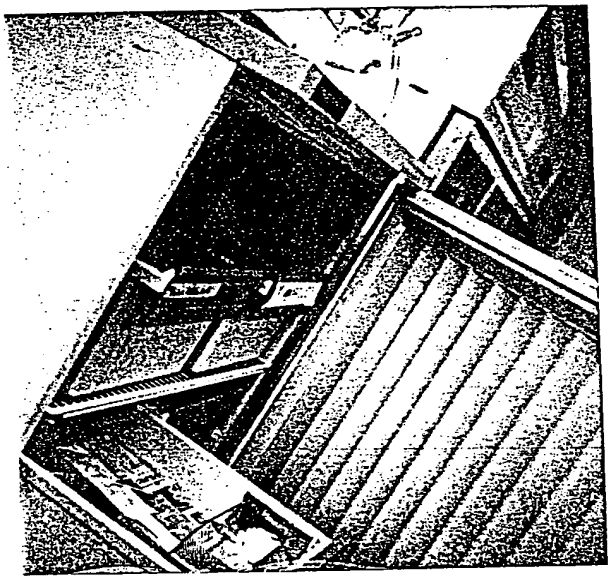
25/70



DeSM1
Ex-10
June 13, 2000 Sa

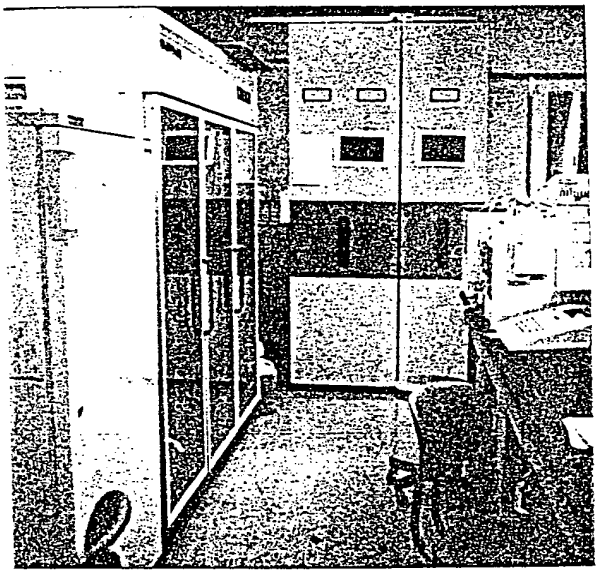


DeSM1
Ex-11
June 13, 2000 Sa



000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K
Ex-13

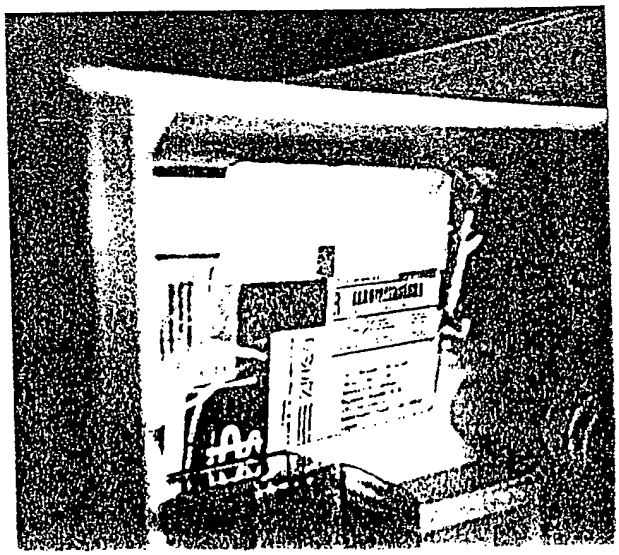
Ex-14 →



000811
DSM4025 stock in refrigeration
Bldg. 27/Rm 54K

Ex-12 →

000811
DSM4025 stock in refrigerator
Bldg. 27/Rm 54K



Declaration SM1
Ex 10 ~ 14

25/70

15

Shinjoh, Masako [NRRC~Tokyo]

差出人: Shinjoh, Masako [NRRC~Tokyo]
送信日時: 2000年8月11日金曜日 午後 3:53
宛先: 'Sales@sawady.com'
CC: Itoh, Naoki [CPO~Tokyo]
件名: PCR-sequencing 依頼

Dec SM1
Ex15

間下さん

いつもお世話になります。
今回の、sequencingもよろしくお願いいたします。
注文の詳細は、弊社伊藤がお伝えしましたように、来週水曜日、8月16日に材料とともにクロネコ宅急便で、17日午前到着指定でおくります。

今回は、支払に係る、こちらの注文番号をお伝えいたします。
注文番号: W0005031

それでは、解析の方、よろしくお願いいたします。

新城雅子

日本ロシュ研究所
所属:応用微生物部
氏名:新城雅子
住所: 鎌倉市梶原200
TEL: 0467-47-2226
PAX:0467-45-6812

26/70

[T3] E-mail from Dr. Shinjoh to Mr. Mashita dated Aug. 11, 2000

From: Shinjoh, Masako

Date: August 11, 2000 3:53 pm

To: Sales@sawady.com

cc: Itoh, Naoki {CPO~Tokyo}

Subject: PCR-sequencing order

Dear Mr. Mashita,

Thank you for your usual service.

I'd like to ask you this sequencing request.

The details of this order and our sample will be sent next Wednesday, Aug. 16, as Mr. Itoh has already let you know via KORONEKO-TAKKYUBIN to arrive to your site in the morning of Aug. 17.

I inform our order number relating the payment;

order number: W0005031.

Could you please perform the analysis.

Best regards,

Masako Shinjoh

NRRC, Applied Microbiology, Masako Shinjoh,

TEL: 0467047-2226 FAX; 0467-45-6812

Declaration SM/
Ex-16

Declaration sm/
Ex-17
28/10

インターネット荷物お問い合わせ http://www.kuronekoyamato.co.jp/

ご依頼主控

お問い合わせ伝票番号
1435-6347-7411

受付 年 月 日

お届け予定(指定)日 月 日

交通事情等により時間帯のお届けご希望に添えない場合もありますのであらかじめご了承下さい。

着店コード

ご希望の方は、お届け時間帯に○をして下さい。

希望なし

午前中
12時)14時
14時)16時
16時)18時
18時)20時
20時)21時

宅急便 00

お届け先
住所 〒03-3788-4633
東京都豊島区南池袋2-9-1
第一池袋ホワイトビル1F
氏名 サヴィー・テクノロジー 御下 様

ご依頼主
住所〒
神奈川県 鎌倉市
梶原 200
日本ロッシュ(株)
研究所
応用微生物部
氏名 新城 雅子 様

品名 サンプル・書類
1. ワレモノ
2. なまもの

宅急便では30万円を超える高価格品はお取扱いできませんので、ご出荷されても損害賠償の責を負いかねます。また危険品、信書、貨幣及び有価証券その他店頭掲示の品目のお取扱いはできません。なお、その他は宅急便約款によります。

備考 TN 6096
C/C 51610

ゴルフ・スキープレー日又は搭乗日 月 日 クラブ本数 本

集荷・持込 現・回・着

60 ②	80 ⑤	100 cm ⑩ kg	
120 ⑮	140 ⑳	160 cm ㉕ kg	
ゴルフ	運賃	円	
スキー			
空港クール		円	
冷凍	合計		
冷蔵		円	

取扱店名又は顧客コード

発店名(コード) 27 - 20

運送料一万円未満

a 1 4 3 5 6 3 4 7 7 4 1 1 a

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中部 0561(61)5000
関西 06(6682)7111
中国 082(849)1500
四国 0877(44)0555
九州 092(931)3300

ヤマト運輸株式会社 東京都中央区銀座2丁目16番10号 本社 03(3541)3411

品番1261(D)

[T1] Declaration SM 1st Exhibit 18; Takkyubin slip

Receiver: Address; Dai-ichi Ikebukuro White Building 1F

2-9-9, Minami-Ikebukuro, Toshima-ku, Tokyo, 171

Phone: 03-3988-4633

Name: Mr. Mashita, Sawady Technology

Sender

Address; Applied Microbiology, Nippon Roche Research Center, 200

Kajiwara Kamakura, Kanagawa.

Name: Masako Shinjoh

Item: Sample

(Unfortunately, copy of this slip was taken before shipping. So, no date on the slip)

Declaration SM 1st-18

E-D

Docket No: C38435/109700CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :	)	
Akira ASAKURA *et al.*	)	Examiner: M. Walicka
Serial No.: 09/470,667	)	Art Unit: 1652
Filed: December 22, 1999	)	
For: **NOVEL ALCOHOL/ALDEHYDE DEHYDROGENASES**	)	

Commissioner for Patents
Washington, DC 20231

SECOND DECLARATION OF DR. MASAKO SHINJOH UNDER 37 C.F.R. § 1.132

Sir:

I, Masako Shinjoh, a citizen and resident of Japan, hereby declare as follows:

1. I am employed by Nippon Roche Research Center of Nippon Roche K.K., Kajiwara 200, Kamakura-shi, Kanagawa-ken 247-8530, Japan (hereafter "NRKK"). I currently hold the position of genetic engineer at NRKK. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. I am a coinventor of U.S. patent application No: 09/470,667 (the '667 application). The '667 application is summarized in more detail in the FIRST DECLARATION OF DR. MASAKO SHINJOH UNDER 37 C.F.R. § 1.132 ("First Declaration") filed concurrently herewith.

3. As described in the First Declaration, after reviewing the Sequence Listing filed with the '667 application, how the nucleotide and amino acid sequences that make up the Sequence Listing were incorporated into the '667 application, and the original nucleotide printouts from the sequencing machine used to read the experimentally derived sequences, I have come to the conclusion that SEQ ID NOs:1, 3, and 7 each contain a single base (SEQ ID NOs:1 and 3) or a single amino acid (SEQ ID NO:7) error that arose through typing errors.

4. After the '667 application was filed, I found discrepancies in the nucleotide and amino acid sequences identified in the '667 application as SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 7, respectively when compared to the computer printouts generated by the nucleotide sequencing machine used to read the nucleotide sequences that ultimately became SEQ ID NOs:1 and 3 in the '667 application. As set forth in more detail below, I believe that each of these discrepancies was the result of a typing mistake made when I prepared the sequence listing data for an internal Research Report.

5. The original sequence data underlying each of the sequences disclosed in the Sequence Listing of the '667 application were generated by a nucleotide sequencing machine, and could not be converted into an electronic file for manipulation in an electronic

medium (*e.g.*, a word processor). Accordingly, I manually typed the sequences ultimately disclosed in the '667 application into an electronic format using the original sequence data generated by the nucleotide sequencing machine. It is my belief that when the original sequence data was retyped into an electronic format that a single base in each of SEQ ID NOs:1 and 3 was entered in error, and that because of the error in SEQ ID NO:3, its deduced amino acid sequence (SEQ ID NO:7) also contained a single amino acid error. The manually re-typed sequences, including the unrecognized typographical mistakes, were then incorporated into the foreign priority application (EP 96115001 filed September 19, 1996), which became the basis for the '667 application including the Sequence Listing contained therein. (Exhibit 2).

6. A copy of the original printout from the nucleotide sequencing machine of the open reading frame of Enzyme A including the nucleotide sequence (which became SEQ ID NO:1 in the '667 application) and its deduced amino acid sequence (which became SEQ ID NO:5 in the '667 application) is attached as Exhibit 3. I have compared the nucleotide and deduced amino acid sequences from the original printout with the sequences disclosed as SEQ ID NOs:1 and 5 in the '667 application, and have found that the nucleotide at position 852 of SEQ ID NO:1 is a "G" whereas the corresponding nucleotide in the original printout is a "C." It is my

belief that the correct nucleotide at position 852 is "C," not "G" as recited in SEQ ID NO:1.

7. Because of the redundancy of the genetic code, when SEQ ID NO:1 was translated, the deduced amino acid encoded by the codon containing the nucleotide at position 852 did not change compared to the deduced amino acid sequence generated by the nucleotide sequencer as set forth in the original printout. Thus, both sequences are identical.

8. A copy of the original printout from the nucleotide sequencing machine of the open reading frame of Enzyme A" including the nucleotide sequence (which became SEQ ID NO:3 in the '667 application) and its deduced amino acid sequence (which became SEQ ID NO:7 in the '667 application) is attached as Exhibit 4. I have compared the nucleotide and deduced amino acid sequences from the original printout with the sequences disclosed as SEQ ID NOs:3 and 7 in the '667 application and have found that the nucleotide at position 644 of SEQ ID NO:3 is an "A" whereas the corresponding nucleotide in the original printout is a "C." It is my belief that the correct nucleotide at position 644 is "C," not "A" as recited in SEQ ID NO:3.

9. The replacement of "A" for "C" at position 644 in SEQ ID NO:3 also led to the translation of a different amino acid ("Asn" was translated

instead of "Thr" at position 192) from the codon containing the error at nucleotide position 644. It is my belief that the correct amino acid at position 192 of SEQ ID NO:7 is "Thr," not "Asn" as currently recited.

10. To verify the correctness of the nucleotide and amino acid sequences identified on the original printouts generated by the nucleotide sequencing machine, which were the bases for the disclosure of SEQ ID NOs:1, 3, and 7 in the '667 application, I obtained a sample of *Gluconobacter oxydans* strain DSM 4025, the same microorganism from which the nucleotide sequences of SEQ ID NOs:1 and 3 were isolated (as disclosed in the '667 application), from the International Depository Authority, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ("DSMZ"), a publicly available cell depository.

11. With the assistance of Mr. Naoki Itoh, NRKK's Patent & Licensing Manager, I then contracted with an independent nucleotide sequencing company (Sawady – see the First Declaration) to use the *Gluconobacter oxydans* DSM 4025 cell sample I obtained from DSMZ to clone and sequence the relevant parts of the chromosomal DNA of these cells.

12. The chain of custody of the cell sample and chromosomal DNA derived therefrom is set forth in my First Declaration and the

DECLARATION OF MR. MASAO MASHITA UNDER 37 C.F.R. § 132 and of the DECLARATION OF MR. YOSHITAKA MURATA UNDER 37 C.F.R. §1.132, both of which are being filed concurrently herewith.

13. With respect to the sequence work, I instructed Sawady to utilize two primer pairs designed by the coinventors for the cloning (by polymerase chain reaction (PCR)) and sequencing of Enzyme A (Primers for Analysis 1) and of Enzyme A" (Primers for Analysis 2) as described below.

Primers for Analysis 1: (for Enzyme A)
Forward: A697f 5'- TACGAAGCCC GTTGGATGAC -3'
Reverse: A1000r 5'- TCGGGTTGAT CGACTGCAGA -3'

Primers for Analysis 2: (for Enzyme A")
Forward: A"479f 5'- TATTCGACGT CGATCGCGGT -3'
Reverse: A"780r 5'- AACTGCTGAG GTGCCGTAGT -3'

14. The Primers for Analysis 1 were designed to amplify (by PCR) the region from nucleotide (nt) position 697 to nt position 1000 of the gene encoding Enzyme A and to determine the amplified nucleotide sequence having 304 bases including the nucleotide at position 852. The primers for Analysis 2 were designed to amplify (by PCR) the region from nt position 479 to nt position 780 of the gene encoding Enzyme A" and to determine the amplified nucleotide sequence having 302 bases including the nucleotide at position 644.

15. The primer information was provided to Mr. Masao Mashita at

Sawady together with a sample of the original microorganism DSM 4025 disclosed in the '667 application (and obtained through DSMZ) to facilitate the cloning and sequencing of the relevant nucleotides for Enzyme A (SEQ ID NO:1) and Enzyme A" (SEQ ID NO:3). (*See* my First Declaration).

16. On October 13, 2000, I received from Sawady, via Mr. Itoh, an Experimental Report (non-finalized) including the sequence data, which are set forth in Exhibit 5. From the anti-parallel alignment of the (+) and (–) strands in combination with the sequence information of the primers used, I confirmed the correctness of the two nucleotide sequences set forth below. For determining each sequence, I took into consideration that at positions downstream of each primer used in the PCR sequencing carried out by Sawady, the nucleotide reading on the sense strand was not absolutely reliable, and thus for each such region, the data from the complementary sequence was used:

Ex. (1): A697-1000 Sawady [304 bp] (corresponds to Enzyme A, *i.e.* SEQ ID NO: 1)

```
697
 TACGAAGCCC GTTGGATGAC CGGTGCCTGG GGCCAGATCA CCTATGACCC

 CGTCACCAAC CTTGTCCACT ACGGCTCGAC CGCTGTGGGT CCGGCGTCGG

 AAACCCAACG CGGCACCCCG GGCGGCACGC TGTACGGCAC GAACACCCGT
    852
 TTCGCCGTGC GTCCTGACAC GGGCGAGATT GTCTGGCGTC ACCAGACCCT

 GCCCCGCGAC AACTGGGACC AGGAATGCAC GTTCGAGATG ATGGTCACCA

 ATGTGGATGT CCAACCCTCG ACCGAGATGG AAGGTCTGCA GTCGATCAAC
```

1000
CCGA

17. The correctness of the above-identified sequence was verified with the two nucleotide sequences (41F903 and 39F903) (Exhibit 5) and two primer sequences (A697f and A1000r):

* Nucleotides 697-716 of Ex(1) above are the same as nucleotides 1-20 of primer A697f;
* Nucleotides 717-966 of Ex(1) above are the same as nucleotides 41-290 of the complementary sequence of 41F903;
* Nucleotides 967-980 of Ex(1) above are the same as nucleotides 253-266 of 39F903; and
* Nucleotides 981-1000 of Ex(1) above are the same as nucleotides 1-20 of the complementary sequence of primer A1000r.

Ex. (2): A"479-780 Sawady [302 bp] (corresponds to Enzyme A", *i.e.* SEQ ID NO: 3)

479
TATTCGACGT CGATCGCGGT CAAGGCACGG ATATGGTCTC GAACTCGTCC

GGCCCGATTG TCGCCAATGG CGTCATCGTT GCGGGCTCGA CCTGTCAGTA

TTCGCCGTTC GGCTGTTTCG TTTCGGGCCA CGACTCGGCC ACCGGTGAAG
644
AGCTGTGGCG CAACACCTTT ATCCCGCGCG CCGGCGAAGA GGGTGATGAG

ACCTGGGGCA ATGATTACGA GGCCCGCTGG ATGACCGGCG TTTGGGGCCA

GATCACCTAT GACCCCGTTG GCGGCCTTGT CCACTACGGC ACCTCAGCAG
780
TT

18. The correctness of the above-identified sequence was verified with two nucleotide sequences (45F903 and 43F903) (Exhibit 5) and two primer sequences (A"479f and A"780r):

* Nucleotides 479-498 of Ex(2) above are the same as nucleotides 1-20 of primer A"479f;
* Nucleotides 499-728 of Ex(2) above are the same as nucleotides 41-270 of the complementary sequence of 45F903;
* Nucleotides 729-760 of Ex(2) above are the same as nucleotides 228-259 of 43F903; and
* Nucleotides 761-780 of Ex(2) above are the same as nucleotides 1-20 of the complementary sequence of primer A"780r.

19. Based on my knowledge and experience, and in view of the results presented herein, it is my opinion that SEQ ID NOs:1 and 3 of the '667 application each contain a single nucleotide error introduced by a typing mistake. The single mistake in SEQ ID NO:1 resulted in no error in the amino acid sequence of SEQ ID NO: 5. The single mistake in SEQ ID NO: 3 when translated resulted in a single amino acid error in SEQ ID NO: 7. Each of these errors is readily identifiable to one of skill in the art by cloning and sequencing the chromosomal DNA of the same microorganism used in the '667 application, which is publicly available. The identification of each of these errors is summarized in more detail below:

(a) SEQ ID NO: 1

By comparing the nucleotide sequence identified above as "A697-1000 Sawady [304 bp]" with the nucleotide sequence recited in the original nucleotide printout from the nucleotide sequencing machine (Exhibit 3), I confirmed that the nucleotides from positions 697-1000 in each sequence are identical. Therefore, the nucleotide at position 852 in SEQ ID NO: 1 ("G") is incorrect and should read "C." This error had no effect on the corresponding deduced amino acid sequence in SEQ ID NO:5.

(b) SEQ ID NO: 3

By comparing the nucleotide sequence identified above as "A"479-780 Sawady [302 bp]" with the nucleotide sequence recited in the original nucleotide printout from the nucleotide sequencing machine (Exhibit 4), I confirmed that the nucleotides from positions 479-780 in each sequence are identical. Therefore, the nucleotide at position 644 in SEQ ID NO: 3 ("A") is incorrect and should read "C."

(c) SEQ ID NO:7

Based on the correct nucleotide sequence for SEQ ID NO: 3, the triplet codon recited as "AAC" of nucleotide positions 643-645 in SEQ ID NO:3 should read "ACC." This should be reflected in the corresponding deduced amino acid sequence (SEQ ID NO: 7) at amino acid position 192, which was recited as "Asn" in the current

Sequence Listing. The correct codon ("ACC"), however, corresponds to the amino acid "Thr." Therefore, the amino acid at position 192 in SEQ ID NO:7 ("Asn") is incorrect and should read "Thr."

20. In sum, on the basis of the data presented herein, resequencing of the relevant parts of the chromosomal DNA of a sample of the same microorganism from which SEQ ID NOs:1 and 3 were isolated as disclosed in the '667 application (*i.e.*, *Gluconobacter oxydans* strain DSM 4025) confirms that typographical mistakes resulted in the following errors found in SEQ ID NOs: 1, 3, and 7, and that such errors would be readily identified by one skilled in this art using publicly available starting materials and routine skill. Accordingly, in my opinion, one skilled in this art would recognize, after resequencing the relevant parts of the chromosomal DNA of DSM 4025 that:

(1) The nucleotide at position 852 of SEQ ID NO:1, which currently recites "G," should recite "C."

(2) The nucleotide at position 644 of SEQ ID NO:3, which currently recites "A," should recite "C."

(3) The amino acid at position 192 of SEQ ID NO:7, which currently recites "Asn," should recite "Thr."

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: September 18, 2002

Masako Shinjoh

Masako Shinjoh

CURRICULUM VITAE of Masako Shinjoh As of August 28, 2002

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200 Kajiwara Kamakura, Japan
247-8530.

Phone: +81-467-47-2226
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Education & Research Experience:

1a. Scientist (April 1979 to date) at Dept of Applied Microbiology, Nippon Roche K.K., Nippon Roche Research Center at Kamakura, Japan, which belongs to Vitamin and Fine Chemical Division in Hoffmann-La Roche

This work includes improvement of microorganisms producing vitamin or its precursor by conventional method and genetic engineering.

1b. Visiting scientist (Jan. to March 1982) at Research Institute of Molecular Biology at Nutley, NJ, USA, which belonged to Hoffmann-La Roche. Objectives: to exchange scientific information and technical transfer of genetic engineering skills.

2. Ph.D. (Jan. 12, 1996)
Ph.D. in Engineering from Department of Fermentation Technology, Osaka University, Osaka, Japan.

The title of the Thesis is "Metabolic engineering for 2-keto-L-gulonic acid production in

Gluconobacter".

3. Master Degree (April 1977 to March 1979)

Master in Engineering from Department of Fermentation Technology, Osaka University, Osaka, Japan.

The projects involved were

"Characterization of bacteriophage of bacitracin-produing *Bacillus*"

"Application of plasmid on fermentation production: factors responsible for stabilization of hybrid plasmids carrying trytophane operon in *E. coli* ."

4. Bachelar Degree (April 1975 to March 1977)

Department of Fermentation Technology, Osaka University, Osaka, Japan.

The projects involved were

"In vitro synthesis of alpha-amylase of *Bacillus*"

5. Professional field

Microbiology
Fermentation technology
Genetic engineering

6. Memberships

a) The Society for Bioscience and Bioengineering
b) Japan Society for Bioscience, Biotechnology, and Agrochemistry

7. Personal information:

Female,
Japanese citizen,
Birthday: 20th February, 1955

LIST OF PUBLICATIONS

Original Papers by the Author

Shinjoh, M., Y. Setoguchi, T. Hoshino and A. Fujiwara. (1990)
L-Sorbose dissimilation in 2-keto-L-gulonic acid-producing mutant UV10 derived from *Gluconobacter melanogenus* IFO 3293. Agric. Biol. Chem. 54: 2257 - 2263.

Shinjoh, M., T. Sugisawa, S. Masuda, and T. Hoshino. (1994)
Efficient conversion of L-sorbosone to 2-keto-L-gulonic acid by *Acetobacter liquefaciens* strains. J. Ferment. Bioeng. 78: 476 - 478.

Shinjoh, M., and T. Hoshino. (1995). Development of a stable shuttle vector and a conjugative transfer system for *Gluconobacter oxydans*.J. Ferment. Bioeng. 79: 95 - 99.

Shinjoh, M., N. Tomiyama, A. Asakura, and T. Hoshino. (1995) Cloning and nucleotide sequencing of membrane-bound L-sorbosone dehydrogenase gene of *Acetobacter liquefaciens* IFO 12258 and its expression in *Gluconobacter oxydans*. Appl. Environ. Microbiol. 43: 1064 - 1069.

Shinjoh, M, M., Tazoe, and T. Hoshino. (2002) NADPH-dependent L-sorbose reductase is responsible for L-sorbose assimilation in *Gluconobacter suboxydans* IFO 3291. J. of Bacteriol., 84: 861 - 863.

Miyazaki, T., N. Tomiyama, M. Shinjoh, and T. Hoshino. (2002) Molecular cloning and functional expression of D-sorbitol dehydrogenase from *Gluconobacter suboxydans* IFO3255 which requires PQQ and hydrophobic protein SldB for the activity development in *E.coli*. (2001) Biosci. Biotechnol. Biochem. 66: 262-270. (the corresponding author)

Shinjoh, M., N. Tomiyama, T. Miyazaki, and T. Hoshino. (2002) Main polyol dehydrogenase of *Gluconobacter suboxydans* IFO 3255, membrane-bound D-sorbitol dehydrogenase, that needs product of upstream gene, *sldB*, for activity. Biosci. Biotechnol. Biochem. (in press)

Other Publications on the work done at Hoffmann-La Roche

Sugisawa, T., T. Hoshino, S. Masuda, S. Nomura, Y. Setoguchi, M. Tazoe, M. Shinjoh, S. Someha and A. Fujiwara. (1990) Microbial production of 2-keto-L-gulonic acid from L-sorbose and D-sorbitol by *Gluconobacter oxydans*. Agric. Biol. Chem. 54: 1201 - 1209.

Hoshino, T., T. Sugisawa, M. Tazoe, M. Shinjoh and A. Fujiwara. (1990) Metabolic pathway for 2-keto-L-gulonic acid formation in *Gluconobacter oxydans* IFO 3293. Agric. Biol. Chem. 54: 1211 - 1218.

Shinjoh, M., (1990) Biotechnology of acetic acid bacteria. Su no kagaku, Asakura shoten. Tokyo. 157 - 170. (in Japanese)

Other Publications on the work done at Osaka Univ.

Imanaka, T., K. Uchida, M. Tateishi (Shinjoh), and S. Aiba. (1979) Inducible bacteriophage of *Bacillus licheniformis* ATCC 10716. Virology 95: 249 - 250.

Tsunekawa, H., M. Tateishi (Shinjoh), T. Imanaka, S. Aiba. (1981) TnA-directed deletion of the trp operon from RSF2124-trp in *Escherichia coli*.

Patent publication: USP granted including "M. Shinjoh" as the inventor

(as of Aug. 28, 2002)

	PAT. NO.	Title
1	6,407,203	Cytochrome *c* and polynucleotides encoding cytochrome *c*
2	6,146,860	Manufacture of L-ascorbic acid and D-erythorbic acid
3	6,127,156	D-sorbitol dehydrogenase gene
4	6,037,147	Cytochrome *c* and polynucleotides encoding cytochrome *c*
5	5,541,108	*Gluconobacter oxydans* strains

6 5,399,496 DNA shuttle vectors for *E. coli*, *Gluconobacter*, and *Acetobacter*

7 5,352,599 Co-enzyme-independent L-sorbosone dehydrogenase of *Gluconobacter oxydans*: isolation, characterization, and cloning and autologus expression of the gene

----------END of CV----------

2nd Declaration Masaki Shinjoh
Exhibit-2

2

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT

NAME: F. HOFFMANN-LA ROCHE AG

STREET: Grezacherstrasse 124

CITY: Basle

COUNTRY: Switzerland

POSTAL CODE: CH-4002

TELEPHONE: 061 - 688 25 11

FAX: 061 - 688 13 95

TELEX: 962292/965542 hlr c

(ii) TITLE OF INVENTION:

Alcohol/Aldehyde dehydrogenase genes

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Macintosh

(C) OPERATING SYSTEM:

(D) SOFTWARE: MS word ver 5.1

(v) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION

(2) **INFORMATION FOR SEQ ID NO:1:**

(i) **SEQUENCE CHARACTERISTICS:**

(A) **LENGTH: 1740 base pairs**

(B) **TYPE: nucleic acid**

(C) **STRANDEDNESS: double**

(D) **TOPOLOGY: linear**

(ii) **MOLECULE TYPE: DNA (genomic)**

(iii) **ORIGINAL SOURCE:**

ORGANISM: ***Gluconobacter oxydans***

STRAIN: DSM 4025

(iv) **FEATURE:**

FEATURE KEY: CDS

POSITIOIN: 1..1737

SEQUENCING METHOD: E

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ATGAAACCGA CTTCGCTGCT TTGGGCCAGT GCTGGCGCAC TTGCATTGCT   50
TGCCGCACCC GCCTTTGCTC AAGTGACCCC CGTCACCGAT GAATTGCTGG  100
CGAACCCGCC CGCTGGTGAA TGGATCAGCT ACGGTCAGAA CCAAGAAAAC  150
TACCGTCACT CGCCCCTGAC GCAGATCACG ACTGAGAACG TCGGCCAACT  200
GCAACTGGTC TGGGCGCGCG GCATGCAGCC GGGCAAAGTC CAAGTCACGC  250
CCCTGATCCA TGACGGCGTC ATGTATCTGG CAAACCCGGG CGACGTGATC  300
CAGGCCATCG ACGCCAAAAC TGGCGATCTG ATCTGGGAAC ACCGCCGCCA  350
ACTGCCGAAC ATCGCCACGC TGAACAGCTT TGGCGAGCCG ACCCGCGGCA  400
TGGCGCTGTA CGGCACCAAC GTTTACTTTG TTTCGTGGGA CAACCACCTG  450
GTCGCCCTCG ACACCGCAAC TGGCCAAGTG ACGTTCGACG TCGACCGCGG  500
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CCAAGGCGAA GACATGGTTT CGAACTCGTC GGGCCCGATC GTGGCAAACG 550

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GTCTCGGGCC ACGACTCGGC CACCGGTGAA GAGCTGTGGC GCAACTACTT 650

CATCCCGCGC GCTGGCGAAG AGGGTGATGA GACTTGGGGC AACGATTACG 700

AAGCCCGTTG GATGACCGGT GCCTGGGGCC AGATCACCTA TGACCCCGTC 750

ACCAACCTTG TCCACTACGG CTCGACCGCT GTGGGTCCGG CGTCGGAAAC 800

CCAACGCGGC ACCCCGGGCG GCACGCTGTA CGGCACGAAC ACCCGTTTCG 850

CGGTGCGTCC TGACACGGGC GAGATTGTCT GGCGTCACCA GACCCTGCCC 900

CGCGACAACT GGGACCAGGA ATGCACGTTC GAGATGATGG TCACCAATGT 950

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TGACCGTGAA CGAAGATGCG ATCCTGAAGG AACTGGATGT TGAATATGAC 1200

GTCTGCCCGA CCTTCTTGGG CGGCCGCGAC TGGCCGTCGG CCGCACTGAA 1250

CCCCGACAGC GGCATCTACT TCATCCCGCT GAACAACGTC TGCTATGACA 1300

TGATGGCCGT CGATCAGGAA TTCACCTCGA TGGACGTCTA TAACACCAGC 1350

AACGTGACCA AGCTGCCGCC CGGCAAGGAT ATGATCGGTC GTATTGACGC 1400

GATCGACATC AGCACGGGTC GTACGCTGTG GTCGGTCGAA CGTGCTGCGG 1450

CGAACTATTC GCCCGTCTTG TCGACCGGCG GCGGCGTTCT GTTCAACGGT 1500

GGTACGGATC GTTACTTCCG CGCCCTCAGC CAAGAAACCG GCGAGACCCT 1550

GTGGCAGACC CGCCTTGCAA CCGTCGCGTC GGGCCAGGCC ATCTCTTACG 1600

AGGTTGACGG CATGCAATAT GTCGCCATCG CAGGTGGTGG TGTCAGCTAT 1650

GGCTCGGGCC TGAACTCGGC ACTGGCTGGC GAGCGAGTCG ACTCGACCGC 1700

CATCGGTAAC GCCGTCTACG TCTTCGCCCT GCCGCAATAA 1740

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1740 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ORIGINAL SOURCE:

ORGANISM: *Gluconobacter oxydans*

STRAIN: DSM 4025

(iv) FEATURE:

FEATURED KEY: CDS

POSITIOIN: 1..1737

SEQUENCING METHOD: E

```
ATGAAGACGT CGTCTTTGCT GGTTGCGAGC GTTGCCGCGC TTGCAAGCTA   50
TAGCTCCTTT GCGCTTGCTC AAGTGACCCC CGTCACCGAT GAATTGCTGG  100
CGAACCCGCC CGCTGGTGAA TGGATCAGCT ACGGTCAGAA CCAAGAAAAC  150
TACCGTCACT CGCCCCTGAC GCAGATCACG ACTGAGAACG TCGGCCAACT  200
GCAACTGGTC TGGGCGCGCG GCATGCAGCC GGGCAAAGTC CAAGTCACGC  250
CCCTGATCCA TGACGGCGTC ATGTATCTGG CAAACCCGGG CGACGTGATC  300
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CAGGCCATCG ACGCCAAAAC TGGCGATCTG ATCTGGGAAC ACCGCCGCCA 350

ACTGCCGAAC ATCGCCACGC TGAACAGCTT TGGCGAGCCG ACCCGCGGCA 400

TGGCGCTGTA CGGCACCAAC GTTTACTTTG TTTCGTGGGA CAACCACCTG 450

GTCGCCCTCG ACACCGCAAC TGGCCAAGTG ACGTTCGACG TCGACCGCGG 500

CCAAGGCGAA GACATGGTTT CGAACTCGTC GGGCCCGATC GTGGCAAACG 550

GCGTGATCGT TGCCGGTTCG ACCTGCCAAT ACTCGCCGTT CGGCTGCTTT 600

GTCTCGGGCC ACGACTCGGC CACCGGTGAA GAGCTGTGGC GCAACTACTT 650

CATCCCGCGC GCTGGCGAAG AGGGTGATGA GACTTGGGGC AACGATTACG 700

AAGCCCGTTG GATGACCGGC GTCTGGGGTC AGATCACCTA TGACCCCGTT 750

GGCGGCCTTG TCCACTACGG CTCGTCGGCT GTTGGCCCGG CTTCGGAAAC 800

CCAGCGCGGC ACCACCGGCG GCACCATGTA CGGCACCAAC ACCCGTTTCG 850

CTGTCCGTCC CGAGACTGGC GAGATCGTCT GGCGTCACCA AACTCTGCCC 900

CGCGACAACT GGGACCAAGA GTGCACCTTC GAGATGATGG TTGCCAACGT 950

TGACGTGCAG CCCGCAGCTG ACATGGACGG CGTCCGCTCG ATCAACCCGA 1000

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GGCACCATGT GGCAGTTCGA CGCCGAAACC GGCGAATTCC TGTGGGCCCG 1100

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GTCTGCCCGC TGCTGCTGGG TGGCCGTGAC TGGCCGTCGG CTGCGCTGAA 1250

CCCCGATACC GGCATCTACT TTATCCCGCT GAACAACACC TGCATGGATA 1300

TCGAAGCTGT CGACCAGGAA TTCAGCTCGC TGGACGTGTA CAACCAAAGC 1350

CTGACCGCCA AAATGGCACC GGGTAAAGAG CTGGTTGGCC GTATCGACGC 1400

CATCGACATC AGCACAGGCC GCACCCTGTG GACCGCTGAG CGCGAAGCCT 1450

CGAACTACGC GCCTGTCCTG TCGACCGCTG GCGGCGTTCT GTTCAACGGC 1500

GGCACCGACC GTTACTTCCG CGCTCTCAGC CAAGAGACCG GCGAGACCCT 1550

GTGGCAGACC CGTCTGGCGA CTGTCGCTTC GGGCCAAGCT GTCTCGTACG 1600

AGATCGACGG CGTCCAATAC ATCGCCATCG GCGGCGGCGG CACGACCTAT 1650

GGTTCGTTCC ACAACCGTCC CCTGGCCGAG CCGGTCGACT CGACCGCGAT 1700

CGGTAATGCG ATGTACGTCT TCGCGCTGCC CCAGCAATAA 1740

INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1737 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ORIGINAL SOURCE:

ORGANISM: *Gluconobacter oxydans*

STRAIN: DSM 4025

(iv) FEATURE:

FEATURE KEY: CDS

POSITIOIN: 1..1734

SEQUENCING METHOD: E

ATGAAACTGA CGACCCTGCT GCAAAGCAGC GCCGCCCTGC TTGTGCTTGG 50

CACCATTCCC GCCCTTGCCC AAACCGCCAT CACCGATGAA ATGCTGGCGA 100

ACCCGCCCGC TGGTGAATGG ATCAACTACG GTCAGAACCA AGAGAACTAC 150

CGCCACTCGC CCCTGACGCA GATTACCGCA GACAACGTCG GCCAACTGCA 200

ACTGGTCTGG GCGCGCGGTA TGGAAGCGGG CAAGATCCAA GTGACCCCGC 250

TTGTCCATGA CGGCGTCATG TATCTGGCAA ACCCCGGTGA CGTGATCCAG 300

GCCATCGACG CCGCGACCGG CGATCTGATC TGGGAACACC GCCGCCAACT 350

GCCGAACATC GCCACGCTGA ACAGCTTTGG TGAGCCGACC CGCGGCATGG 400

CCCTCTATGG CACCAACGTC TATTTCGTCT CGTGGGACAA CCACTTGGTC 450

GCGCTGGACA CCTCGACCGG CCAAGTCGTA TTCGACGTCG ATCGCGGTCA 500

AGGCACGGAT ATGGTCTCGA ACTCGTCCGG CCCGATTGTC GCCAATGGCG 550

TCATCGTTGC GGGCTCGACC TGTCAGTATT CGCCGTTCGG CTGTTTCGTT 600

TCGGGCCACG ACTCGGCCAC CGGTGAAGAG CTGTGGCGCA ACAACTTTAT 650

CCCGCGCGCC GGCGAAGAGG GTGATGAGAC CTGGGGCAAT GATTACGAGG 700

CCCGCTGGAT GACCGGCGTT TGGGGCCAGA TCACCTATGA CCCCGTTGGC 750

GGCCTTGTCC ACTACGGCAC CTCAGCAGTT GGCCCTGCGG CCGAGATTCA 800

GCGCGGCACC GTTGGCGGCT CGATGTATGG CACCAACACC CGCTTTGCTG 850

TCCGCCCCGA GACCGGCGAG ATCGTCTGGC GTCACCAAAC TCTGCCCCGC 900

GACAACTGGG ACCAAGAGTG TACGTTCGAG ATGATGGTCG TCAACGTCGA 950

CGTCCAGCCC TCGGCTGAGA TGGAAGGCCT GCACGCCATC AACCCCGATG 1000

CCGCCACGGG CGAGCGTCGC GTTGTGACCG GCGTTCCGTG CAAGAACGGC 1050

ACCATGTGGC AGTTCGACGC CGAAACCGGC GAATTCCTGT GGGCGCGCGA 1100

CACCAGCTAT CAGAACCTGA TCGAAAGCGT CGATCCCGAT GGTCTGGTGC 1150

ATGTGAACGA AGATCTGGTC GTGACCGAGC TGGAAGTGGC CTATGAAATC 1200

TGCCCGACCT TCCTGGGTGG CCGCGACTGG CCGTCGGCTG CGCTGAACCC 1250

CGATACTGGC ATCTATTTCA TCCCGCTGAA CAACGCCTGT AGCGGTATGA 1300

CGGCTGTCGA CCAAGAGTTC AGCTCGCTCG ATGTGTATAA CGTCAGCCTC 1350

GACTATAAAC TGTCGCCCGG TTCGGAAAAC ATGGGCCGTA TCGACGCCAT 1400

CGACATCAGC ACCGGCCGCA CGCTGTGGTC GGCTGAACGC TACGCCTCGA 1450

ACTACGCGCC TGTCCTGTCC ACCGGCGGCG GCGTGCTGTT CAACGGCGGC 1500

ACCGACCGTT ACTTCCGCGC CCTCAGCCAA GAGACCGGCG AGACGCTGTG 1550

GCAGACCCGT CTGGCGACTG TCGCCTCGGG TCAAGCGATT TCCTATGAGA 1600

TCGACGGCGT GCAATATGTC GCCATCGGGC GCGGCGGCAC CAGCTATGGC 1650

AGCAACCACA ACCGCGCCCT GACCGAGCGG ATCGACTCGA CCGCCATCGG 1700

CAGCGCGATC TATGTCTTTG CTCTGCCGCA GCAGTAA 1737

INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1740 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ORIGINAL SOURCE:

ORGANISM: *Gluconobacter oxydans*

STRAIN: DSM 4025

(iv) FEATURE:

FEATURE KEY: CDS

POSITIOIN: 1..1737

SEQUENCING METHOD: E

ATGAACCCCA CAACGCTGCT TCGCACCAGC GCGGCCGTGC TATTGCTTAC 50

CGCGCCCGCC GCATTCGCGC AGGTAACCCC GATTACCGAT GAACTGCTGG 100

CGAACCCGCC CGCTGGTGAA TGGATTAACT ACGGCCGCAA CCAAGAAAAC 150

TATCGCCACT CGCCCCTGAC CCAGATCACT GCCGACAACG TTGGTCAGTT 200

GCAACTGGTC TGGGCCCGCG GGATGGAGGC GGGGGCCGTA CAGGTCACGC 250

CGATGATCCA TGATGGCGTG ATGTATCTGG CAAACCCCGG TGATGTGATC 300

CAGGCGCTGG ATGCGCAAAC AGGCGATCTG ATCTGGGAAC ACCGCCGCCA 350

ACTGCCCGCC GTCGCCACGC TAAACGCCCA AGGCGACCGC AAGCGCGGCG 400

TCGCCCTTTA CGGCACGAGC CTCTATTTCA GCTCATGGGA CAACCATCTG 450

ATCGCGCTGG ATATGGAGAC GGGCCAGGTC GTATTCGATG TCGAACGTGG 500

ATCGGGCGAA GACGGCTTGA CCAGTAACAC CACGGGGCCG ATTGTCGCCA 550

ATGGCGTCAT CGTCGCGGGT TCCACCTGCC AATATTCGCC CTATGGATGC 600

TTTATCTCGG GGCACGATTC CGCGACGGGT GAGGAGCTGT GGCGCAACCA 650

CTTTATCCCG CAGCCGGGCG AAGAGGGTGA CGAGACTTGG GGCAATGATT 700

TCGAGGCGCG CTGGATGACC GGCGTCTGGG GTCAGATCAC CTATGATCCC 750

GTGACGAACC TTGTGTTCTA TGGCTCGACC GGCGTGGGCC CAGCGTCCGA 800

AACCCAGCGC GGCACGCCGG GCGGCACGCT GTATGGCACC AACACCCGCT 850

TTGCGGTGCG TCCCGACACG GGCGAGATTG TCTGGCGTCA CCAGACCCTG 900

CCGCGCGACA ACTGGGACCA AGAATGCACG TTCGAGATGA TGGTCGCCAA 950

CGTCGATGTG CAACCCTCGG CCGAGATGGA GGGTCTGCGC GCCATCAACC 1000

CCAATGCGGC GACGGGCGAG CGCCGTGTGC TGACGGGTGC GCCTTGCAAG 1050

ACCGGCACGA TGTGGTCGTT TGATGCGGCC TCGGGCGAAT TCCTGTGGGC 1100

GCGTGATACC AACTACACCA ATATGATCGC CTCGATCGAC GAGACCGGCC 1150

TTGTGACGGT GAACGAGGAT GCGGTGCTGA AAGAGCTGGA CGTTGAATAT 1200

GACGTCTGCC CGACCTTCCT GGGTGGGCGC GACTGGTCGT CAGCCGCACT 1250

GAACCCGGAC ACCGGCATTT ACTTCTTGCC GCTGAACAAT GCCTGCTACG 1300

ATATTATGGC CGTTGATCAA GAGTTTAGCG CGCTCGACGT CTATAACACC 1350

AGCGCGACCG CAAAACTCGC GCCGGGCTTT GAAAATATGG GCCGCATCGA 1400

CGCGATTGAT ATCAGCACCG GGCGCACCTT GTGGTCGGCG GAGCGCCCTG 1450

CGGCGAACTA CTCGCCCGTT TTGTCGACGG CAGGCGGTGT GGTGTTCAAC 1500

GGCGGGACCG ACCGCTATTT CCGTGCCCTC AGCCAGGAAA CCGGCGAGAC 1550

TTTGTGGCAG GCCCGTCTTG CGACGGTCGC GACGGGGCAG GCGATCAGCT 1600

ACGAGTTGGA CGGCGTGCAA TATATCGCCA TCGGTGCGGG CGGTCTGACC 1650

TATGGCACGC AATTGAACGC GCCGCTGGCC GAGGCAATCG ATTCGACCTC 1700

GGTCGGTAAT GCGATCTATG TCTTTGCACT GCCGCAGTAA 1740

INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 579 residues

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:

ORGANISM: *Gluconobacter oxydans*

STRAIN: DSM 4025

(iv) FEATURE:

FEATURE KEY: sig peptide

POSITION: -23..-1

SEQUENCING METHOD: E

FEATURE KEY: mat peptide

POSITION: 1..556

SEQUENCING METHOD: E

```
Met Lys Pro Thr Ser Leu Leu Trp Ala Ser Ala Gly Ala Leu Ala
  -20                 -15                 -10

Leu Leu Ala Ala Pro Ala Phe Ala Gln Val Thr Pro Val Thr Asp
  -5                   1                   5

Glu Leu Leu Ala Asn Pro Pro Ala Gly Glu Trp Ile Ser Tyr Gly
 10                  15                  20

Gln Asn Gln Glu Asn Tyr Arg His Ser Pro Leu Thr Gln Ile Thr
 25                  30                  35

Thr Glu Asn Val Gly Gln Leu Gln Leu Val Trp Ala Arg Gly Met
 40                  45                  50

Gln Pro Gly Lys Val Gln Val Thr Pro Leu Ile His Asp Gly Val
 55                  60                  65

Met Tyr Leu Ala Asn Pro Gly Asp Val Ile Gln Ala Ile Asp Ala
 70                  75                  80

Lys Thr Gly Asp Leu Ile Trp Glu His Arg Arg Gln Leu Pro Asn
 85                  90                  95

Ile Ala Thr Leu Asn Ser Phe Gly Glu Pro Thr Arg Gly Met Ala
100                 105                 110

Leu Tyr Gly Thr Asn Val Tyr Phe Val Ser Trp Asp Asn His Leu
115                 120                 125

Val Ala Leu Asp Thr Ala Thr Gly Gln Val Thr Phe Asp Val Asp
130                 135                 140
```

Arg Gly Gln Gly Glu Asp Met Val Ser Asn Ser Ser Gly Pro Ile
145 150 155

Val Ala Asn Gly Val Ile Val Ala Gly Ser Thr Cys Gln Tyr Ser
160 165 170

Pro Phe Gly Cys Phe Val Ser Gly His Asp Ser Ala Thr Gly Glu
175 180 185

Glu Leu Trp Arg Asn Tyr Phe Ile Pro Arg Ala Gly Glu Glu Gly
190 195 200

Asp Glu Thr Trp Gly Asn Asp Tyr Glu Ala Arg Trp Met Thr Gly
205 210 215

Ala Trp Gly Gln Ile Thr Tyr Asp Pro Val Thr Asn Leu Val His
220 225 230

Tyr Gly Ser Thr Ala Val Gly Pro Ala Ser Glu Thr Gln Arg Gly
235 240 245

Thr Pro Gly Gly Thr Leu Tyr Gly Thr Asn Thr Arg Phe Ala Val
250 255 260

Arg Pro Asp Thr Gly Glu Ile Val Trp Arg His Gln Thr Leu Pro
265 270 275

Arg Asp Asn Trp Asp Gln Glu Cys Thr Phe Glu Met Met Val Thr
280 285 290

Asn Val Asp Val Gln Pro Ser Thr Glu Met Glu Gly Leu Gln Ser
295 300 305

Ile Asn Pro Asn Ala Ala Thr Gly Glu Arg Arg Val Leu Thr Gly
310 315 320

Val Pro Cys Lys Thr Gly Thr Met Trp Gln Phe Asp Ala Glu Thr
325 330 335

Gly Glu Phe Leu Trp Ala Arg Asp Thr Asn Tyr Gln Asn Met Ile
340 345 350

Glu Ser Ile Asp Glu Asn Gly Ile Val Thr Val Asn Glu Asp Ala
355 360 365

Ile Leu Lys Glu Leu Asp Val Glu Tyr Asp Val Cys Pro Thr Phe
370 375 380

Leu Gly Gly Arg Asp Trp Pro Ser Ala Ala Leu Asn Pro Asp Ser

385 390 395

Gly Ile Tyr Phe Ile Pro Leu Asn Asn Val Cys Tyr Asp Met Met
400 405 410

Ala Val Asp Gln Glu Phe Thr Ser Met Asp Val Tyr Asn Thr Ser
415 420 425

Asn Val Thr Lys Leu Pro Pro Gly Lys Asp Met Ile Gly Arg Ile
430 435 440

Asp Ala Ile Asp Ile Ser Thr Gly Arg Thr Leu Trp Ser Val Glu
445 450 455

Arg Ala Ala Ala Asn Tyr Ser Pro Val Leu Ser Thr Gly Gly Gly
460 465 470

Val Leu Phe Asn Gly Gly Thr Asp Arg Tyr Phe Arg Ala Leu Ser
475 480 485

Gln Glu Thr Gly Glu Thr Leu Trp Gln Thr Arg Leu Ala Thr Val
490 495 500

Ala Ser Gly Gln Ala Ile Ser Tyr Glu Val Asp Gly Met Gln Tyr
505 510 515

Val Ala Ile Ala Gly Gly Gly Val Ser Tyr Gly Ser Gly Leu Asn
520 525 530

Ser Ala Leu Ala Gly Glu Arg Val Asp Ser Thr Ala Ile Gly Asn
535 540 545

Ala Val Tyr Val Phe Ala Leu Pro Gln
550 555

INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 579 residues

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:

ORGANISM: ***Gluconobacter oxydans***

STRAIN: DSM 4025

(iv) FEATURE:

FEATURE KEY: sig peptide

POSITION: -23..-1

SEQUENCING METHOD: S

FEATURE KEY: mat peptide

POSITION: 1..556

SEQUENCING METHOD: S

```
Met Lys Thr Ser Ser Leu Leu Val Ala Ser Val Ala Ala Leu Ala
          -20                 -15                 -10

Ser Tyr Ser Ser Phe Ala Leu Ala Gln Val Thr Pro Val Thr Asp
          -5                  1                   5

Glu Leu Leu Ala Asn Pro Pro Ala Gly Glu Trp Ile Ser Tyr Gly
        10                  15                  20

Gln Asn Gln Glu Asn Tyr Arg His Ser Pro Leu Thr Gln Ile Thr
        25                  30                  35

Thr Glu Asn Val Gly Gln Leu Gln Leu Val Trp Ala Arg Gly Met
        40                  45                  50

Gln Pro Gly Lys Val Gln Val Thr Pro Leu Ile His Asp Gly Val
        55                  60                  65
```

Met Tyr Leu Ala Asn Pro Gly Asp Val Ile Gln Ala Ile Asp Ala
70 75 80

Lys Thr Gly Asp Leu Ile Trp Glu His Arg Arg Gln Leu Pro Asn
85 90 95

Ile Ala Thr Leu Asn Ser Phe Gly Glu Pro Thr Arg Gly Met Ala
100 105 110

Leu Tyr Gly Thr Asn Val Tyr Phe Val Ser Trp Asp Asn His Leu
115 120 125

Val Ala Leu Asp Thr Ala Thr Gly Gln Val Thr Phe Asp Val Asp
130 135 140

Arg Gly Gln Gly Glu Asp Met Val Ser Asn Ser Ser Gly Pro Ile
145 150 155

Val Ala Asn Gly Val Ile Val Ala Gly Ser Thr Cys Gln Tyr Ser
160 165 170

Pro Phe Gly Cys Phe Val Ser Gly His Asp Ser Ala Thr Gly Glu
175 180 185

Glu Leu Trp Arg Asn Tyr Phe Ile Pro Arg Ala Gly Glu Glu Gly
190 195 200

Asp Glu Thr Trp Gly Asn Asp Tyr Glu Ala Arg Trp Met Thr Gly
205 210 215

Val Trp Gly Gln Ile Thr Tyr Asp Pro Val Gly Gly Leu Val His
220 225 230

Tyr Gly Ser Ser Ala Val Gly Pro Ala Ser Glu Thr Gln Arg Gly
235 240 245

Thr Thr Gly Gly Thr Met Tyr Gly Thr Asn Thr Arg Phe Ala Val
250 255 260

Arg Pro Glu Thr Gly Glu Ile Val Trp Arg His Gln Thr Leu Pro
265 270 275

Arg Asp Asn Trp Asp Gln Glu Cys Thr Phe Glu Met Met Val Ala
280 285 290

Asn Val Asp Val Gln Pro Ala Ala Asp Met Asp Gly Val Arg Ser
295 300 305

Ile Asn Pro Asn Ala Ala Thr Gly Glu Arg Arg Val Leu Thr Gly

310 315 320

Val Pro Cys Lys Thr Gly Thr Met Trp Gln Phe Asp Ala Glu Thr
325 330 335

Gly Glu Phe Leu Trp Ala Arg Asp Thr Ser Tyr Glu Asn Ile Ile
340 345 350

Glu Ser Ile Asp Glu Asn Gly Ile Val Thr Val Asp Glu Ser Lys
355 360 365

Val Leu Thr Glu Leu Asp Thr Pro Tyr Asp Val Cys Pro Leu Leu
370 375 380

Leu Gly Gly Arg Asp Trp Pro Ser Ala Ala Leu Asn Pro Asp Thr
385 390 395

Gly Ile Tyr Phe Ile Pro Leu Asn Asn Thr Cys Met Asp Ile Glu
400 405 410

Ala Val Asp Gln Glu Phe Ser Ser Leu Asp Val Tyr Asn Gln Ser
415 420 425

Leu Thr Ala Lys Met Ala Pro Gly Lys Glu Leu Val Gly Arg Ile
430 435 440

Asp Ala Ile Asp Ile Ser Thr Gly Arg Thr Leu Trp Thr Ala Glu
445 450 455

Arg Glu Ala Ser Asn Tyr Ala Pro Val Leu Ser Thr Ala Gly Gly
460 465 470

Val Leu Phe Asn Gly Gly Thr Asp Arg Tyr Phe Arg Ala Leu Ser
475 480 485

Gln Glu Thr Gly Glu Thr Leu Trp Gln Thr Arg Leu Ala Thr Val
490 495 500

Ala Ser Gly Gln Ala Val Ser Tyr Glu Ile Asp Gly Val Gln Tyr
505 510 515

Ile Ala Ile Gly Gly Gly Gly Thr Thr Tyr Gly Ser Phe His Asn
520 525 530

Arg Pro Leu Ala Glu Pro Val Asp Ser Thr Ala Ile Gly Asn Ala
535 540 545

Met Tyr Val Phe Ala Leu Pro Gln Gln
550 555

INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 578 residues

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:

ORGANISM: *Gluconobacter oxydans*

STRAIN: DSM 4025

(iv) FEATURE:

FEATURE KEY: sig peptide

POSITION: -23..-1

SEQUENCING METHOD: S

FEATURE KEY: mat peptide

POSITION: 1..555

SEQUENCING METHOD: S

```
Met Lys Leu Thr Thr Leu Leu Gln Ser Ser Ala Ala Leu Leu Val
          -20                 -15                 -10

Leu Gly Thr Ile Pro Ala Leu Ala Gln Thr Ala Ile Thr Asp Glu
```

```
          -5                  1                   5

Met Leu Ala Asn Pro Pro Ala Gly Glu Trp Ile Asn Tyr Gly Gln
        10                  15                  20

Asn Gln Glu Asn Tyr Arg His Ser Pro Leu Thr Gln Ile Thr Ala
        25                  30                  35

Asp Asn Val Gly Gln Leu Gln Leu Val Trp Ala Arg Gly Met Glu
        40                  45                  50

Ala Gly Lys Ile Gln Val Thr Pro Leu Val His Asp Gly Val Met
        55                  60                  65

Tyr Leu Ala Asn Pro Gly Asp Val Ile Gln Ala Ile Asp Ala Ala
        70                  75                  80

Thr Gly Asp Leu Ile Trp Glu His Arg Arg Gln Leu Pro Asn Ile
        85                  90                  95

Ala Thr Leu Asn Ser Phe Gly Glu Pro Thr Arg Gly Met Ala Leu
       100                 105                 110

Tyr Gly Thr Asn Val Tyr Phe Val Ser Trp Asp Asn His Leu Val
       115                 120                 125

Ala Leu Asp Thr Ser Thr Gly Gln Val Val Phe Asp Val Asp Arg
       130                 135                 140

Gly Gln Gly Thr Asp Met Val Ser Asn Ser Ser Gly Pro Ile Val
       145                 150                 155

Ala Asn Gly Val Ile Val Ala Gly Ser Thr Cys Gln Tyr Ser Pro
       160                 165                 170

Phe Gly Cys Phe Val Ser Gly His Asp Ser Ala Thr Gly Glu Glu
       175                 180                 185

Leu Trp Arg Asn Asn Phe Ile Pro Arg Ala Gly Glu Glu Gly Asp
       190                 195                 200

Glu Thr Trp Gly Asn Asp Tyr Glu Ala Arg Trp Met Thr Gly Val
       205                 210                 215

Trp Gly Gln Ile Thr Tyr Asp Pro Val Gly Gly Leu Val His Tyr
       220                 225                 230

Gly Thr Ser Ala Val Gly Pro Ala Ala Glu Ile Gln Arg Gly Thr
       235                 240                 245
```

Val Gly Gly Ser Met Tyr Gly Thr Asn Thr Arg Phe Ala Val Arg
250 255 260

Pro Glu Thr Gly Glu Ile Val Trp Arg His Gln Thr Leu Pro Arg
265 270 275

Asp Asn Trp Asp Gln Glu Cys Thr Phe Glu Met Met Val Val Asn
280 285 290

Val Asp Val Gln Pro Ser Ala Glu Met Glu Gly Leu His Ala Ile
295 300 305

Asn Pro Asp Ala Ala Thr Gly Glu Arg Arg Val Val Thr Gly Val
310 315 320

Pro Cys Lys Asn Gly Thr Met Trp Gln Phe Asp Ala Glu Thr Gly
325 330 335

Glu Phe Leu Trp Ala Arg Asp Thr Ser Tyr Gln Asn Leu Ile Glu
340 345 350

Ser Val Asp Pro Asp Gly Leu Val His Val Asn Glu Asp Leu Val
355 360 365

Val Thr Glu Leu Glu Val Ala Tyr Glu Ile Cys Pro Thr Phe Leu
370 375 380

Gly Gly Arg Asp Trp Pro Ser Ala Ala Leu Asn Pro Asp Thr Gly
385 390 395

Ile Tyr Phe Ile Pro Leu Asn Asn Ala Cys Ser Gly Met Thr Ala
400 405 410

Val Asp Gln Glu Phe Ser Ser Leu Asp Val Tyr Asn Val Ser Leu
415 420 425

Asp Tyr Lys Leu Ser Pro Gly Ser Glu Asn Met Gly Arg Ile Asp
430 435 440

Ala Ile Asp Ile Ser Thr Gly Arg Thr Leu Trp Ser Ala Glu Arg
445 450 455

Tyr Ala Ser Asn Tyr Ala Pro Val Leu Ser Thr Gly Gly Gly Val
460 465 470

Leu Phe Asn Gly Gly Thr Asp Arg Tyr Phe Arg Ala Leu Ser Gln
475 480 485

Glu Thr Gly Glu Thr Leu Trp Gln Thr Arg Leu Ala Thr Val Ala
490 495 500

```
Ser Gly Gln Ala Ile Ser Tyr Glu Ile Asp Gly Val Gln Tyr Val
      505                 510                 515

Ala Ile Gly Arg Gly Gly Thr Ser Tyr Gly Ser Asn His Asn Arg
      520                 525                 530

Ala Leu Thr Glu Arg Ile Asp Ser Thr Ala Ile Gly Ser Ala Ile
      535                 540                 545

Tyr Val Phe Ala Leu Pro Gln Gln
      550                 555
```

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 579 residues

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:

ORGANISM: *Gluconobacter oxydans*

STRAIN: DSM 4025

(iv) FEATURE:

FEATURE KEY: sig peptide

POSITION: -23..-1

SEQUENCING METHOD: E

FEATURE KEY: mat peptide

POSITION: 1..556

SEQUENCING METHOD: E

```
Met Asn Pro Thr Thr Leu Leu Arg Thr Ser Ala Ala Val Leu Leu
 -20                 -15                 -10

Leu Thr Ala Pro Ala Ala Phe Ala Gln Val Thr Pro Ile Thr Asp
 -5                   1                   5

Glu Leu Leu Ala Asn Pro Pro Ala Gly Glu Trp Ile Asn Tyr Gly
 10                  15                  20

Arg Asn Gln Glu Asn Tyr Arg His Ser Pro Leu Thr Gln Ile Thr
 25                  30                  35

Ala Asp Asn Val Gly Gln Leu Gln Leu Val Trp Ala Arg Gly Met
 40                  45                  50

Glu Ala Gly Ala Val Gln Val Thr Pro Met Ile His Asp Gly Val
 55                  60                  65

Met Tyr Leu Ala Asn Pro Gly Asp Val Ile Gln Ala Leu Asp Ala
 70                  75                  80

Gln Thr Gly Asp Leu Ile Trp Glu His Arg Arg Gln Leu Pro Ala
 85                  90                  95

Val Ala Thr Leu Asn Ala Gln Gly Asp Arg Lys Arg Gly Val Ala
 100                 105                 110

Leu Tyr Gly Thr Ser Leu Tyr Phe Ser Ser Trp Asp Asn His Leu
 115                 120                 125

Ile Ala Leu Asp Met Glu Thr Gly Gln Val Val Phe Asp Val Glu
 130                 135                 140

Arg Gly Ser Gly Glu Asp Gly Leu Thr Ser Asn Thr Thr Gly Pro
 145                 150                 155

Ile Val Ala Asn Gly Val Ile Val Ala Gly Ser Thr Cys Gln Tyr
 160                 165                 170

Ser Pro Tyr Gly Cys Phe Ile Ser Gly His Asp Ser Ala Thr Gly
```

175 180 185

Glu Glu Leu Trp Arg Asn His Phe Ile Pro Gln Pro Gly Glu Glu
190 195 200

Gly Asp Glu Thr Trp Gly Asn Asp Phe Glu Ala Arg Trp Met Thr
205 210 215

Gly Val Trp Gly Gln Ile Thr Tyr Asp Pro Val Thr Asn Leu Val
220 225 230

Phe Tyr Gly Ser Thr Gly Val Gly Pro Ala Ser Glu Thr Gln Arg
235 240 245

Gly Thr Pro Gly Gly Thr Leu Tyr Gly Thr Asn Thr Arg Phe Ala
250 255 260

Val Arg Pro Asp Thr Gly Glu Ile Val Trp Arg His Gln Thr Leu
265 270 275

Pro Arg Asp Asn Trp Asp Gln Glu Cys Thr Phe Glu Met Met Val
280 285 290

Ala Asn Val Asp Val Gln Pro Ser Ala Glu Met Glu Gly Leu Arg
295 300 305

Ala Ile Asn Pro Asn Ala Ala Thr Gly Glu Arg Arg Val Leu Thr
310 315 320

Gly Ala Pro Cys Lys Thr Gly Thr Met Trp Ser Phe Asp Ala Ala
325 330 335

Ser Gly Glu Phe Leu Trp Ala Arg Asp Thr Asn Tyr Thr Asn Met
340 345 350

Ile Ala Ser Ile Asp Glu Thr Gly Leu Val Thr Val Asn Glu Asp
355 360 365

Ala Val Leu Lys Glu Leu Asp Val Glu Tyr Asp Val Cys Pro Thr
370 375 380

Phe Leu Gly Gly Arg Asp Trp Ser Ser Ala Ala Leu Asn Pro Asp
385 390 395

Thr Gly Ile Tyr Phe Leu Pro Leu Asn Asn Ala Cys Tyr Asp Ile
400 405 410

Met Ala Val Asp Gln Glu Phe Ser Ala Leu Asp Val Tyr Asn Thr
415 420 425

Ser Ala Thr Ala Lys Leu Ala Pro Gly Phe Glu Asn Met Gly Arg
430 435 440

Ile Asp Ala Ile Asp Ile Ser Thr Gly Arg Thr Leu Trp Ser Ala
445 450 455

Glu Arg Pro Ala Ala Asn Tyr Ser Pro Val Leu Ser Thr Ala Gly
460 465 470

Gly Val Val Phe Asn Gly Gly Thr Asp Arg Tyr Phe Arg Ala Leu
475 480 485

Ser Gln Glu Thr Gly Glu Thr Leu Trp Gln Ala Arg Leu Ala Thr
490 495 500

Val Ala Thr Gly Gln Ala Ile Ser Tyr Glu Leu Asp Gly Val Gln
505 510 515

Tyr Ile Ala Ile Gly Ala Gly Gly Leu Thr Tyr Gly Thr Gln Leu
520 525 530

Asn Ala Pro Leu Ala Glu Ala Ile Asp Ser Thr Ser Val Gly Asn
535 540 545

Ala Ile Tyr Val Phe Ala Leu Pro Gln
550 555

INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 bases

(B) TYPE: nucleotide

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) ORIGINAL SOURCE: synthetic oligonucleotide

```
CATGAAAATA AAAACAGGTG CACGCATCCT CGCATTATCC GCATTAACGA 50
CGATGATGTT TTCCGCCTCG GCTCTCGCCC AG                    82
```

INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 83 bases

(B) TYPE: nucleotide

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) ORIGINAL SOURCE: synthetic oligonucleotide

```
GTTACCTGGG CGAGAGCCGA GGCGGAAAAC ATCATCGTCG TTAATGCGGA 50
TAATGCGAGG ATGCGTGCAC CTGTTTTTAT TTT                   83
```

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 residues

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) ORIGINAL SOURCE: *E. coli*

(iv) FEATURE:

FEATURE KEY: sig peptide

POSITION: 1..26

FEATURE METHOD: S

```
Met Lys Ile Lys Thr Gly Ala Arg Ile Leu Ala Leu Ser Ala Leu
 1               5                  10                  15

Thr Thr Met Met Phe Ser Ala Ser Ala Leu Ala Gln
            20                  25     27
```

INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 bases

(B) TYPE: nucleotide

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) ORIGINAL SOURCE: synthetic oligonucleotide

GTTAGCGCGG TGGATCCCCA TTGGAGG 27

*** GENIAS - Nucleotide Usage Table ***

DNA Sequence Name (1): pEnzA (1-1740)
Comment :

T	319	(18.3%)
C	559	(32.1%)
A	356	(20.5%)
G	506	(29.1%)
Total	[1740]	

[G+C]% = 61.2 %

*** GENIAS - Codon Usage Table ***
Frame No. 1

DNA Sequence Name (1): pEnzA (1-1740)
Comment :

TTT	Phe	4	0.7%	TCT	Ser	1	0.2%	TAT	Tyr	8	1.4%	TGT	Cys	0	0.0%
TTC	Phe	13	2.2%	TCC	Ser	1	0.2%	TAC	Tyr	14	2.4%	TGC	Cys	6	1.0%
TTA	Leu	0	0.0%	TCA	Ser	0	0.0%	TAA	***	1	0.2%	TGA	***	0	0.0%
TTG	Leu	4	0.7%	TCG	Ser	23	4.0%	TAG	***	0	0.0%	TGG	Trp	16	2.8%
CTT	Leu	5	0.9%	CCT	Pro	1	0.2%	CAT	His	1	0.2%	CGT	Arg	11	1.9%
CTC	Leu	2	0.3%	CCC	Pro	11	1.9%	CAC	His	6	1.0%	CGC	Arg	13	2.2%
CTA	Leu	0	0.0%	CCA	Pro	0	0.0%	CAA	Gln	14	2.4%	CGA	Arg	1	0.2%
CTG	Leu	29	5.0%	CCG	Pro	18	3.1%	CAG	Gln	13	2.2%	CGG	Arg	0	0.0%
ATT	Ile	2	0.3%	ACT	Thr	6	1.0%	AAT	Asn	1	0.2%	AGT	Ser	1	0.2%
ATC	Ile	24	4.1%	ACC	Thr	33	5.7%	AAC	Asn	31	5.3%	AGC	Ser	7	1.2%
ATA	Ile	0	0.0%	ACA	Thr	0	0.0%	AAA	Lys	4	0.7%	AGA	Arg	0	0.0%
ATG	Met	16	2.8%	ACG	Thr	12	2.1%	AAG	Lys	3	0.5%	AGG	Arg	0	0.0%
GTT	Val	8	1.4%	GCT	Ala	7	1.2%	GAT	Asp	11	1.9%	GGT	Gly	16	2.8%
GTC	Val	26	4.5%	GCC	Ala	23	4.0%	GAC	Asp	24	4.1%	GGC	Gly	44	7.6%
GTA	Val	0	0.0%	GCA	Ala	11	1.9%	GAA	Glu	21	3.6%	GGA	Gly	0	0.0%
GTG	Val	12	2.1%	GCG	Ala	9	1.6%	GAG	Glu	12	2.1%	GGG	Gly	0	0.0%

Total [580]

Dc SM2 Ex3 ½

Nov. 16 '06

A-1

41/70

Dc SM2 EX3 2/2

A-2

4/20

*** GENIAS - Amino Acid Translation **

DNA Sequence Name (1): pEnzA (1-1740)
Comment :

> Genetic Code [Universal]

```
        10         20         30         40         50         60         70         80         90        100        110        120
ATGAAACCGACTTCGCTGCTTTGGGCCAGTGCTGGCGCACTTGCATTGCTTGCCGCACCCGCCTTTGCTCAAGTGACCCCCGTCACCGATGAATTGCTGGCGAACCCGCCCGCTGGTGAA
M  K  P  T  S  L  L  W  A  S  A  G  A  L  A  L  L  A  A  P  A  F  A  Q  V  T  P  V  T  D  E  L  L  A  N  P  P  A  G  E

       130        140        150        160        170        180        190        200        210        220        230        240
TGGATCAGCTACGGTCAGAACCAAGAAAACTACCGTCACTCGCCCCCTGACGCAGATCACGACTGAGAACGTCGGCCAACTGCAACTGGTCTGGGGCGCGCGGCATGCAGCCGGGCAAAGTC
W  I  S  Y  G  Q  N  Q  E  N  Y  R  H  S  P  L  T  Q  I  T  T  E  N  V  G  Q  L  Q  L  V  W  A  R  G  M  Q  P  G  K  V

       250        260        270        280        290        300        310        320        330        340        350        360
CAAGTCACGCCCCTGATCCATGACGGGCGTCATGTATCTGGCAAACCCGGGCGACGTGATCCAGGCCATCGACGCCAAAACTGGCGATCTGATCTGGGAACACCGCCGCCAACTGCCGAAC
Q  V  T  P  L  I  H  D  G  V  M  Y  L  A  N  P  G  D  V  I  Q  A  I  D  A  K  T  G  D  L  I  W  E  H  R  R  Q  L  P  N

       370        380        390        400        410        420        430        440        450        460        470        480
ATCGCCACGCTGAACAGCTTTGGCGAGCCGACCCGCGGCATGGCGCTGTACGGCACCAACGTTTACTTTGTTTCGTGGGACAACCACCTGGTCGCCCTCGACACCGCAACTGGCCAAGTG
I  A  T  L  N  S  F  G  E  P  T  R  G  M  A  L  Y  G  T  N  V  Y  F  V  S  W  D  N  H  L  V  A  L  D  T  A  T  G  Q  V

       490        500        510        520        530        540        550        560        570        580        590        600
ACGTTCGACGTCGACCGCGGCCAAGGCGAAGACATGGTTTCGAACTCGTCGGGCCCGATCGTGGCAAACGGCGTGATCGTTGCCGGTTCGACCTGCCAATACTCGCCGTTCGGCTGCTTT
T  F  D  V  D  R  G  Q  G  E  D  M  V  S  N  S  S  G  P  I  V  A  N  G  V  I  V  A  G  S  T  C  Q  Y  S  P  F  G  C  F

       610        620        630        640        650        660        670        680        690        700        710        720
GTCTCGGGCCACGACTCGGCCACCGGTGAAGAGCTGTGGCGCAACTACTTCATCCCGCGCGCTGGCGAAGAGGGTGATGAGACTTGGGGCAACGATTACGAAGCCCGTTGGATGACCGGT
V  S  G  H  D  S  A  T  G  E  E  L  W  R  N  Y  F  I  P  R  A  G  E  E  G  D  E  T  W  G  N  D  Y  E  A  R  W  M  T  G

       730        740        750        760        770        780        790        800        810        820        830        840
GCCTGGGGCCAGATCACCTATGACCCCGTCACCAACCTTGTCCACTACGGCTCGACCGCTGTGGGTCCGGCGTCGGAAACCCAACGCGGCACCCCGGGCGGCACGCTGTACGGCACGAAC
A  V  G  Q  I  T  Y  D  P  V  T  N  L  V  H  Y  G  S  T  A  V  G  P  A  S  E  T  Q  R  G  T  P  G  G  T  L  Y  G  T  N

       850        860        870        880        890        900        910        920        930        940        950        960
ACCCGTTTCGCCGTGCGTCCTGACACGGGCGAGATTGTCTGGCGTCACCAGACCCTGCCCCGCGACAACTGGGACCAGGAATGCACGTTCGAGATGATGGTCACCAATGTGGATGTCCAA
T  R  F  A  V  R  P  D  T  G  E  I  V  W  R  H  Q  T  L  P  R  D  N  W  D  Q  E  C  T  F  E  M  M  V  T  N  V  D  V  Q

       970        980        990       1000       1010       1020       1030       1040       1050       1060       1070       1080
CCCTCGACCGAGATGGAAGGTCTGCAGTCGATCAACCCGAACGCCGCAACTGGCGAGCGTCGCGTGCTGACCGGCGTTCCGTGCAAAACCGGCACCATGTGGCAGTTCGACGCCGAAACC
P  S  T  E  M  E  G  L  Q  S  I  N  P  N  A  A  T  G  E  R  R  V  L  T  G  V  P  C  K  T  G  T  M  W  Q  F  D  A  E  T

       1090       1100       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
GGCGAATTCCTGTGGGCCCGTGATACCAACTACCAGAACATGATCGAATCCATCGACGAAAACGGCATCGTGACCGTGAACGAAGATGCGATCCTGAAGGAACTGGATGTTGAATATGAC
G  E  F  L  W  A  R  D  T  N  Y  Q  N  M  I  E  S  I  D  E  N  G  I  V  T  V  N  E  D  A  I  L  K  E  L  D  V  E  Y  D

       1210       1220       1230       1240       1250       1260       1270       1280       1290       1300       1310       1320
GTCTGCCCGACCTTCTTGGGCGGCCGCGACTGGCCGTCGGCCGCACTGAACCCCGACAGCGGCATCTACTTCATCCCGCTGAACAACGTCTGCTATGACATGATGGCCGTCGATCAGGAA
V  C  P  T  F  L  G  G  R  D  W  P  S  A  A  L  N  P  D  S  G  I  Y  F  I  P  L  N  N  V  C  Y  D  M  M  A  V  D  Q  E

       1330       1340       1350       1360       1370       1380       1390       1400       1410       1420       1430       1440
TTCACCTCGATGGACGTCTATAACACCAGCAACGTGACCAAGCTGCCGCCCGGCAAGGATATGATCGGTCGTATTGACGCGATCGACATCAGCACGGGTCGTACGCTGTGGTCGGTCGAA
F  T  S  M  D  V  Y  N  T  S  N  V  T  K  L  P  P  G  K  D  M  I  G  R  I  D  A  I  D  I  S  T  G  R  T  L  W  S  V  E

       1450       1460       1470       1480       1490       1500       1510       1520       1530       1540       1550       1560
CGTGCTGCGGCGAACTATTCGCCCGTCTTGTCGACCGGCGGCGGCGTTCTGTTCAACGGTGGTACGGATCGTTACTTCCGCGCCCTCAGCCAAGAAACCGGCGAGACCCTGTGGCAGACC
R  A  A  A  N  Y  S  P  V  L  S  T  G  G  G  V  L  F  N  G  G  T  D  R  Y  F  R  A  L  S  Q  E  T  G  E  T  L  W  Q  T

       1570       1580       1590       1600       1610       1620       1630       1640       1650       1660       1670       1680
CGCCTTGCAACCGTCGCGTCGGGCCAGGCCATCTCTTACGAGGTTGACGGCATGCAATATGTCGCCATCGCAGGTGGTGGTGTCAGCTATGGCTCGGGCCTGAACTCGGCACTGGCTGGC
R  L  A  T  V  A  S  G  Q  A  I  S  Y  E  V  D  G  M  Q  Y  V  A  I  A  G  G  G  V  S  Y  G  S  G  L  N  S  A  L  A  G

       1690       1700       1710       1720       1730       1740
GAGCGAGTCGACTCGACCGCCATCGGTAACGCCGTCTACGTCTTCGCCCTGCCGCAATAA
E  R  V  D  S  T  A  I  G  N  A  V  Y  V  F  A  L  P  Q  *
```

4

A'' A''-1

4/5/89 Sn MG→AM 43/70

Dc Sm2 Ex4 1/2

*** GENIAS - Amino Acid Translation **

DNA Sequence Name (1): ENZA''   (1-2284)
Comment   :

> Genetic Code [Universal]

```
                                                 TGCCATAGCCCATCGCGCCCAGCGACAGCGCCGATACGCGCAGCCCTTGGCCAAGTTCACGGGTGA

TCATGGTGCAACTCCCCTCTCTGTGCCGTTTGCCGTTAGGATTAAAGGCATCTGGACGCAGGATGACAATATCATCTGGCGGCTAGGGCCTATGCGGGATCAGGATCAATCGCCATGCAA

GTCATGCAATCATTTTAGGCACTTAAAACTTGCGAATCGCGACAATTTGCCCCAGCGTGCTTTTGTCTCATGCGTATCTTCTCGGAGGAGAGGGATGCGTTTATGCGTTGGAGGACAGAG

       10        20        30        40        50        60        70        80        90       100       110       120
ATGAAACTGACGACCCTGCTGCAAAGCAGCGCCGCCCTGCTTGTGCTTGGCACCATTCCCGCCCTTGCCCAAACCGCCATCACCGATGAAATGCTGGCGAACCCGCCCGCTGGTGAATGG
M  K  L  T  T  L  L  Q  S  S  A  A  L  L  V  L  G  T  I  P  A  L  A  Q  T  A  I  T  D  E  M  L  A  N  P  P  A  G  E  W

      130       140       150       160       170       180       190       200       210       220       230       240
ATCAACTACGGTCAGAACCAAGAGAACTACCGCCACTCGCCCCTGACGCAGATTACCGCAGACAACGTCGGCCAACTGCAACTGGTCTGGGCGCGCGGTATGGAAGCGGGCAAGATCCAA
I  N  Y  G  Q  N  Q  E  N  Y  R  H  S  P  L  T  Q  I  T  A  D  N  V  G  Q  L  Q  L  V  W  A  R  G  M  E  A  G  K  I  Q

      250       260       270       280       290       300       310       320       330       340       350       360
GTGACCCCGCTTGTCCATGACGGCGTCATGTATCTGGCAAACCCCGGTGACGTGATCCAGGCCATCGACGCCGCGACCGGCGATCTGATCTGGGAACACCGCCGCCAACTGCCGAACATC
V  T  P  L  V  H  D  G  V  M  Y  L  A  N  P  G  D  V  I  Q  A  I  D  A  A  T  G  D  L  I  W  E  H  R  R  Q  L  P  N  I

      370       380       390       400       410       420       430       440       450       460       470       480
GCCACGCTGAACAGCTTTGGTGAGCCGACCCGCGGCATGGCCCTCTATGGCACCAACGTCTATTTCGTCTCGTGGGACAACCACTTGGTCGCGCTGGACACCTCGACCGGCCAAGTCGTA
A  T  L  N  S  F  G  E  P  T  R  G  M  A  L  Y  G  T  N  V  Y  F  V  S  W  D  N  H  L  V  A  L  D  T  S  T  G  Q  V  V

      490       500       510       520       530       540       550       560       570       580       590       600
TTCGACGTCGATCGCGGTCAAGGCACGGATATGGTCTCGAACTCGTCCGGCCCGATTGTCGCCAATGGCGTCATCGTTGCGGGCTCGACCTGTCAGTATTCGCCGTTCGGCTGTTTCGTT
F  D  V  D  R  G  Q  G  T  D  M  V  S  N  S  S  G  P  I  V  A  N  G  V  I  V  A  G  S  T  C  Q  Y  S  P  F  G  C  F  V

      610       620       630       640       650       660       670       680       690       700       710       720
TCGGGCCACGACTCGGCCACCGGTGAAGAGCTGTGGCGCAACACCTTTATCCCGCGCGCCGGCGAAGAGGGTGATGAGACCTGGGGCAATGATTACGAGGCCCGCTGGATGACCGGCGTT
S  G  H  D  S  A  T  G  E  E  L  W  R  N (T) F  I  P  R  A  G  E  E  G  D  E  T  W  G  N  D  Y  E  A  R  W  M  T  G  V
                                          =, Noisy

      730       740       750       760       770       780       790       800       810       820       830       840
TGGGGCCAGATCACCTATGACCCCGTTGGCGGCCTTGTCCACTACGGCACCTCAGCAGTTGGCCCTGCGGCCGAGATTCAGCGCGGCACCGTTGGCGGCTCGATGTATGGCACCAACACC
W  G  Q  I  T  Y  D  P  V  G  G  L  V  H  Y  G  T  S  A  V  G  P  A  A  E  I  Q  R  G  T  V  G  G  S  M  Y  G  T  N  T

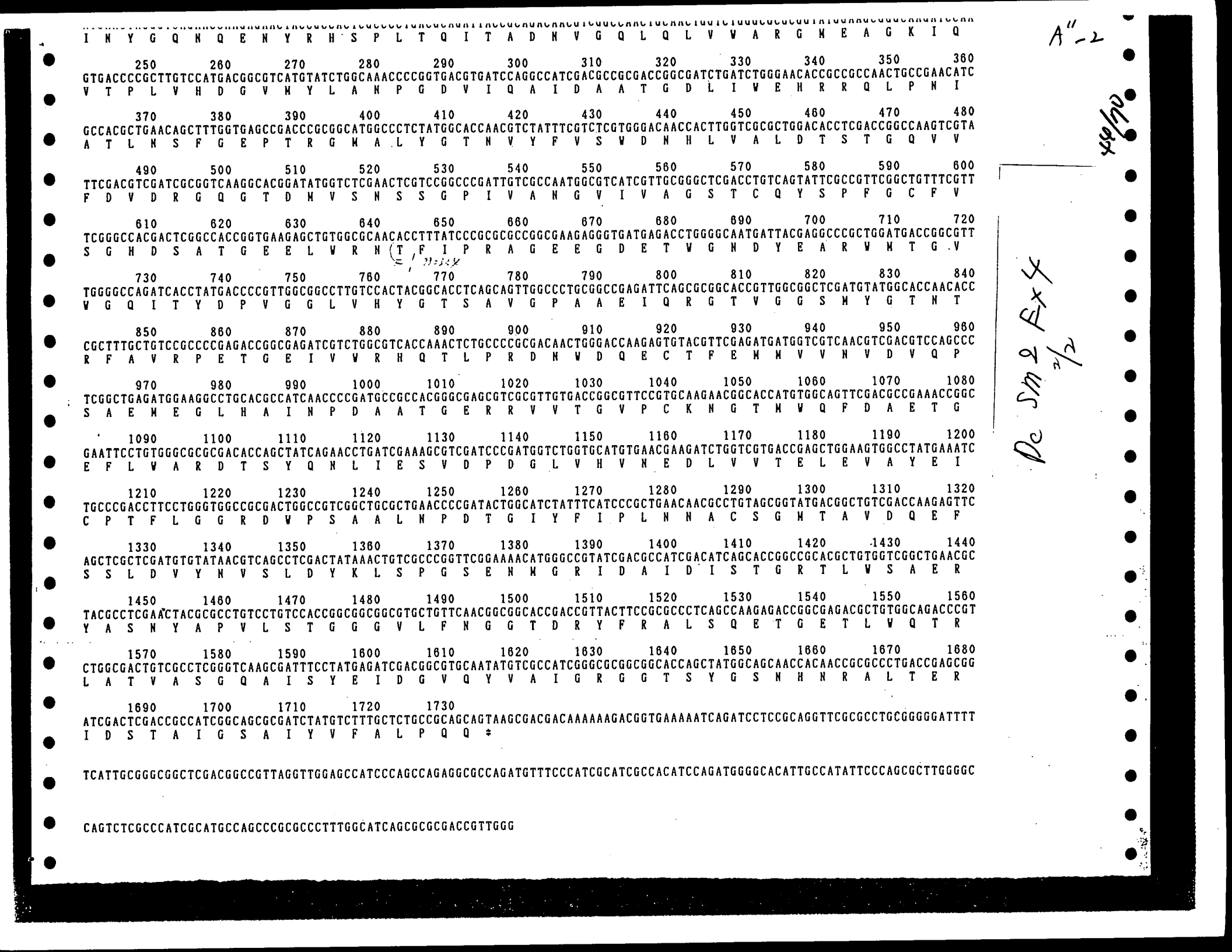
      850       860       870       880       890       900       910       920       930       940       950       960
CGCTTTGCTGTCCGCCCCGAGACCGGCGAGATCGTCTGGCGTCACCAAACTCTGCCCCGCGACAACTGGGACCAAGAGTGTACGTTCGAGATGATGGTCGTCAACGTCGACGTCCAGCCC
R  F  A  V  R  P  E  T  G  E  I  V  W  R  H  Q  T  L  P  R  D  N  W  D  Q  E  C  T  F  E  M  M  V  V  N  V  D  V  Q  P

      970       980       990      1000      1010      1020      1030      1040      1050      1060      1070      1080
TCGGCTGAGATGGAAGGCCTGCACGCCATCAACCCCGATGCCGCCACGGGCGAGCGTCGCGTTGTGACCGGCGTTCCGTGCAAGAACGGCACCATGTGGCAGTTCGACGCCGAAACCGGC
S  A  E  M  E  G  L  H  A  I  N  P  D  A  A  T  G  E  R  R  V  V  T  G  V  P  C  K  N  G  T  M  W  Q  F  D  A  E  T  G

     1090      1100      1110      1120      1130      1140      1150      1160      1170      1180      1190      1200
GAATTCCTGTGGGCGCGCGACACCAGCTATCAGAACCTGATCGAAAGCGTCGATCCCGATGGTCTGGTGCATGTGAACGAAGATCTGGTCGTGACCGAGCTGGAAGTGGCCTATGAAATC
E  F  L  W  A  R  D  T  S  Y  Q  N  L  I  E  S  V  D  P  D  G  L  V  H  V  N  E  D  L  V  V  T  E  L  E  V  A  Y  E  I

     1210      1220      1230      1240      1250      1260      1270      1280      1290      1300      1310      1320
TGCCCGACCTTCCTGGGTGGCCGCGACTGGCCGTCGGCTGCGCTGAACCCCGATACTGGCATCTATTTCATCCCGCTGAACAACGCCTGTAGCGGTATGACGGCTGTCGACCAAGAGTTC
C  P  T  F  L  G  G  R  D  W  P  S  A  A  L  N  P  D  T  G  I  Y  F  I  P  L  N  N  A  C  S  G  M  T  A  V  D  Q  E  F

     1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430      1440
```



Attachment (B)

Sequences of the amplified products.

39F903 (697-1000)/A697f.Seq

TTNCGTGCCT GGGGCCAGAT CACCTATGAC CCCGTCACCA ACCTTGTCCA
CTACGGCTCG ACCGCTGTGG GTCCGGCGTC GGAAACCCAA CGCGGCACCC
CGGGCGGCAC GCTGTACGGC ACGAACACCC GTTTCGCCGT GCGTCCTGAC
ACGGGCGAGA TTGTCTGGCG TCACCAGACC CTGCCCCGCG ACAACTGGGA
CCAGGAATGC ACGTTCGAGA TGATGGTCAC CAATGTGGAT GTCCAACCCT
CGACCGAGAT GGAAGGTCTG CAGTCGATCA ANCGAAANNN NNNNNNNNNN
NNNNN

41F903 (697-1000)/A1000r.Seq

TTCCTCTTGG TCGAGGGTTG GACATCCACA TTGGTGACCA TCATCTCGAA
CGTGCATTCC TGGTCCCAGT TGTCGCGGGG CAGGGTCTGG TGACGCCAGA
CAATCTCGCC CGTGTCAGGA CGCACGGCGA AACGGGTGTT CGTGCCGTAC
AGCGTGCCGC CCGGGGTGCC GCGTTGGGTT TCCGACGCCG GACCCACAGC
GGTCGAGCCG TAGTGGACAA GGTTGGTGAC GGGGTCATAG GTGATCTGGC
CCCAGGCACC GGTCATCCAA CGGGCTTTGT AANNNNNNNN NNNNNNNNNN
N

43F903 (479-780)/A479f.Seq

AAAGCACTTT ATGGNCTCGA ACTCTCCGGC CCGATTGTCG CCAATGGCGT
CATCGTTGCG GGCTCGACCT GTCAGTATTC GCCGTTCGGC TGTTTCGTTT
CGGGCCACGA CTCGGCCACC GGTGAAGAGC TGTGGCGCAA CACCTTTATC
CCGCGCGCCG GCGAAGAGGG TGATGAGACC TGGGGCAATG ATTACGAGGC
CCGCTGGATG ACCGGCGTTT GGGGCCAGAT CACCTATGAC CCCGTTGGCG
GCCTTGTCCA CTACGGCACC TCAAGAGTTA ANANNNNNNN NNNNNNNNN

45F903 (479-780)/A780r.Seq

GACAAGGCTN NCACGGNGTC ATAGGTGATN TGGCCCCAAA CGCCGGTCAT
CCAGCGGGCC TCGTAATCAT TGCCCCAGGT CTCATCACCC TCTTCGCCGG
CGCGCGGGAT AAAGGTGTTG CGCCACAGCT CTTCACCGGT GGCCGAGTCG
TGGCCCGAAA CGAAACAGCC GAACGGCGAA TACTGACAGG TCGAGCCCGC
AACGATGACG CCATTGGCGA CAATCGGGCC GGACGAGTTC GAGACCATAT
CCGTGCCTTG ACCGCGATCG ACGTCCATAA ANNNNNNNNN NNNNNNNNNN

EX. E

Docket No: C38435/109700CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :	)	
Akira ASAKURA *et al.*	)	Examiner: M. Walicka
Serial No.: 09/470,667	)	Art Unit: 1652
Filed: December 22, 1999	)	
For: **NOVEL ALCOHOL/ALDEHYDE DEHYDROGENASES**	)	

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF MR. YOSHITAKA MURATA UNDER 37 C.F.R. § 1.132

Sir:

I, Yoshitaka Murata, a citizen and resident of Japan, hereby declare as follows:

1. I am employed by K.K. Kyurin Corporation, 27-25, Morishita-cho, Yahatanishi-ku, Kitakyushu-shi, Fukuoka-ken, 806-0046 Japan ("Kyurin"). I hold the position of Scientist (Kyurin Omtest Laboratory Dept. "KOLA") at Kyurin. One of my duties at Kyurin is to coordinate the preparation of chromosomal DNA from various cell lines in response to orders from clients of Kyurin. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. By way of background, Kyurin is an independent commercial entity that is not affiliated with the Nippon Roche Research Center of

Nippon Roche K.K. ("NRKK"). Among the services provided by Kyurin to its clients is the preparation of chromosomal DNA from various kinds of cell lines. It is in this capacity that I was contacted by Mr. Masao Mashita of Sawady Technology Co., Ltd., 1-29-10, Maeno-cho, Itabashi-ku, Tokyo, Japan 174-0063 ("Sawady") regarding our ability and interest in preparing chromosomal DNA by reconstituting and growing up a lyophilized sample of *Gluconobacter oxydans* DSM 4025 as set forth in more detail below.

3. At the beginning of August, 2000, I was asked by Mr. Mashita to have Kyurin reconstitute, grow up, and harvest chromosomal DNA from a lyophilized sample of *Gluconobacter oxydans* DSM 4025 cells that he would provide to me.

4. On August 10, Mr. Mashita sent a letter to Kyurin via facsimile (a copy of the original facsimile in Japanese is attached as Exhibit 2 and its translation in English is attached as Exhibit 3). This letter confirmed our agreement with Sawady that Kyurin would conduct the requested work and included an "ORDER FORM" (original written in Japanese, a copy of which is attached as Exhibit 4; its English translation is attached as Exhibit 5) and a set of "GENERAL PROTOCOLS" (original written in English, Exhibit 6; its Japanese translation as Exhibit 7) describing the methods to be used by us for isolating the requested chromosomal DNA.

5. On August 18, 2000, I received a package from Mr. Mashita via overnight courier. The package contained an ampoule identified as containing lyophilized cells of *Gluconobacter oxydans* DSM 4025 and an order sheet from Sawady (original written in Japanese, a copy of which is attached as Exhibit 8; its English translation is attached as Exhibit 9).

6. As soon as I received the package, I stored the package in a refrigerator accessible only to authorized Kyurin personnel at 4°C. Later that day, Dr. Sugama, Director, KOLA Kyurin, at my direction, sent an e-mail to Mr. Mashita to confirm receipt of the ampoule and the order letter.

7. On August 26, 2000, I gave Ms. Masako Nomaguchi, a researcher employed by Kyurin, the ampoule I received from Mr. Mashita on August 18, 2000, identified as containing lyophilized cells of *Gluconobacter oxydans* DSM 4025, and instructed Ms. Nomaguchi to reconstitute the lyophilized cells contained in the ampoule, to grow up those cells, and to isolate chromosomal DNA from those cells.

8. On August 31, 2000, Ms. Nomaguchi informed me that she had completed isolating the chromosomal DNA from the cells grown up from the *Gluconobacter oxydans* DSM 4025 sample I had given her, which I had received from Mr. Mashita on August 18, 2000. Ms.

Nomaguchi collected the isolated chromosomal DNA in a 1.5ml tube, which was labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831."

9. On August 31, 2000, I placed the 1.5ml tube labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831" containing the isolated chromosomal DNA prepared by Ms. Nomaguchi into a shipping package. That same day, I forwarded to Mr. Mashita the package containing the tube labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831" containing the chromosomal DNA isolated from the *Gluconobacter oxydans* DSM 4025 cells prepared by Ms. Nomaguchi.

10. In sum, the ampoule containing the lyophilized *Gluconobacter oxydans* DSM 4025 cells that I received from Mr. Mashita on August 18, 2000 was the same ampoule that I gave to Ms. Nomaguchi, who reconstituted the cells, grew them up, and isolated genomic DNA from them. And, the chromosomal DNA that Ms. Nomaguchi isolated from the reconstituted *Gluconobacter oxydans* DSM 4025 cells was the same DNA contained in the tube labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831" that I forwarded to Mr. Mashita on August 31, 2000.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: September 11, 2002

Yoshitaka Murata

Yoshitaka Murata

CURRICULUM VITAE of Yoshitaka Murata

Scientist
K.K. Kyurin Corporation,
Address 27-25, Morishita-cho, Yahatanishi-ku, Kitakyushu-shi, Fukuoka-ken, 806-0046 Japan

Phone: +81-93-642-3911
FAX: +81-93-642-3967
E-mail: kola@kyurin.co.jp

Education & Research Experience:

1. Present Title
Scientist at K. K. Kyurin Corporation

2. Master Degree (April 1994 to March 1996)
Master in Chemistry from Department of Chemistry, Faculty of Science, Fukuoka University, Japan.
The title of Master thesis:
"Involvement of Cytoskeletal Proteins in the Membrane Stability of Human Erythrocytes under Hydrostatic Pressure" instructed by Prof. Shigeyuki Terada

3. Bachelor Degree (April 1990 to March 1994)
Department of Chemistry, Faculty of Science, Fukuoka University, Japan.
The title of Bachelor thesis:
"Effect of distribution of phospholipids in the Membrane on the Hemolysis of Human Erythrocytes under Hydrostatic Pressure" instructed by Prof. Eiji Kimoto

4. Professional field
Molecular biology, Genetic engineering

5. Memberships

a) Japanese Society for Immunology

6. Personal information:

Male

Japanese citizen,

Birthday: July 7, 1970

LIST OF PUBLICATIONS

Original Papers

Yamaguchi T, Murata Y, Kobayashi J, Kimoto E. (1994) Effects of chemical modification of membrane thiol groups on hemolysis of human erythrocytes under hydrostatic pressure. **Biochim Biophys Acta 1195: 205-10**

----------END of CV----------

received
Aug. 17, 2002
M. Shingo

FAX送信表

平成12年 8月10日

送信先	KYURIN MEDICAL LABORATORY KOLA (KYURIN Omtest Laboratory Dept.) 研究員 野間口 雅子 株式会社キューリン 北九州市八幡西区森下町27番25号 〒806-0046 TEL093-642-3911㈹ FAX093-642-3967
発信元	〒171 東京都豊島区南池袋2−9−9 第1池袋ホワイトビル1F (株)サワディー テクノロジー PHONE 03-3988-4633 FAX 03-3982-5666

用 件／新規オーダー

前略

先日 洲鎌さんへ連絡した件ですが、正式に発注になりました。菌体からプロモゾンをDNA抽出1、RT-PCR 迄をお願いします。概算で結構ですので、御見積をお願いします。

草々

送信枚数 本表含め 4枚

担当者 野間口

Declaration Y. Murata
Exhibit-2

17/26

58/70

[T4] FAX from Mr. Mashita to Kyurin, KOLA dated Aug. 10, 2000

To: KYURIN, KOLA (KYURIN Omtest Laboratory Dept.)
Researcher Masako Nomaguchi
K.K. Kyurin Corporation, 27-25, Morishita-cho, Yahatanishi-ku,
Kitakyushu-shi, Fukuoka-ken, 806-0046
TEL 093-642-3911(Representative) FAX 093-642-3967

From: K.K. Sawady Technology
Dai-ichi Ikebukuro White Building 1F
2-9-9, Minami-Ikebukuro, Toshima-ku, Tokyo, 171
PHONE 03-3988-4633 FAX 03-3982-5666

Date: August 10, 2000

Subject: New order

Hello,

The subject I informed Mr. Sugama was officially ordered. Please extract chromosomal DNA from cells and do RT-PCR*. Please let me know approximate estimate.

Best regards,

Masako Mashita

Declaration K. Murata
Exh. 6, 7 - 3

株式会社サワデーテクノロジー御中
遺伝子塩基配列解析依頼書
(発注)

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1. 依頼日: ２０００年８月　日

Declaration K. Murata
Exhibit-4 (1/2)

2. 依頼者

氏名: 星 野 達 雄
所属: 応用微生物部
日本ロシュ研究所 日本ロシュ株式会社
所在地: 〒247-8530 神奈川県鎌倉市梶原 200
TEL: 0467-47-2226 (Ext. 3116) **FAX**: 0467-45-6812
(お問い合わせ先：新城雅子, Ph.D／**e-mail**: masako.shinjoh@roche.com)

3. 解析結果送付先： 依頼者直送

4. サンプル解析方法
PCR 生成物の直接配列決定 [添付の GENERAL PROTOCOLS をご参照下さい]

5. オプション: [８.その他の項をご参照下さい]

6. 解析サンプル記述欄
解析１： 名称 Enzyme A *(nt 697 - 1000);
解析塩基数 ３０４ｂｐ
解析２： 名称 Enzyme A" *(nt 479 - 780)
解析塩基数 ３０２ｂｐ
(PCR Primer を含めて PCR 生成物の全体について配列決定願います)
[注 *：Enzyme A 及び Enzyme A"は、米国特許出願番号 09/470,667 の優先権主張の基礎となるヨーロッパ特許出願 EP96115001.8 に記述されているように、ゲッチンゲン（ドイツ）の Deutsche Sammlung von Mikroorganismen に寄託されている菌株 DSM 4025 から、依頼人らが遺伝子を同定し、クローニングし、配列決定した新規なアルコール／アルデヒドデヒドロゲナーゼである。]

7. PCR Primer について

解析 1 の Primer

Forward:	A697f:	5'- TACGAAGCCC GTTGGATGAC - 3' (GC 11/20)
Reverse:	A1000r:	5'- TCGGGTTGAT CGACTGCAGA - 3' (GC 11/20)

解析 2 の Primer

Forward:	A"479f:	5'- TATTCGACGT CGATCGCGGT - 3' (GC 11/20)
Reverse:	A"780r:	5'- AACTGCTGAG GTGCCGTAGT - 3' (GC 11/20)

8. その他

[1] Order letter (依頼状、英文 2 部)をご確認戴き、日付及びサインをされたうえ、依頼人宛て 1 通を返送してください。

[2] お送りしました菌株（strain DSM 4025）を添付の培地にて起こし、育成願います。

[3] 上記菌株から chromosomal DNA を調製し、上記解析 1 及び解析 2 のそれぞれの Primer にて増幅してください。

[4] 上記解析 1 及び解析 2 に記載の通りの各 PCR product の配列決定に加え、実験方法、条件及び結果を詳細に記述した実験報告書（英文、実験責任者の日付とサインにより発効されたもの）の作成をお願いします。なお、後日実験責任者の方に宣誓供述書（Declaration）の作成にご協力戴きたく存じます。

以上

Declaration of Murata
Exhibit-4 (continued) (4/2)

Declaration Y. Murata
Exhibit-5 (1/2)

[Translation of ORDER FORM]

Sawady Technology Co., LTD.
ORDER FORM for Analysis of Genetic Base Sequence
(Order)

---

1. **Order Date** August 2000

2. **Order person**

Name: Tatsuo Hoshino, Dr.
Organization: Department of Applied Microbiology,
Nippon Roche Research Center, Nippon Roche K.K.
Address 200 Kajiwara, Kamakura-shi, Kanagawa-ken, 247-8530 Japan
TEL : 0467-47-2226 (Ext. 3116) **FAX :**0467-45-6812
(Contact person: Dr. Masako Shinjoh, **e-mail :** masako.shinjoh@roche.com)

3. **Report is addressed to:** Order person directly

4. **Analysis Methods of Sample:**
PCR product direct sequencing [See attached GENERAL PROTOCOLS]

5. **Option :** [See: the description 8. Others below]

6. **Description on the samples to be analyzed:**

Analysis 1: Name Enzyme A* (nt 697 - 1000);
Number of nucleotides 304 bp
Analysis 2: Name Enzyme A" * (nt 479 - 780)
Number of nucleotides 302 bp

(whole PCR products should be sequenced with the primers for PCR)

[Note *: Enzyme A and Enzyme A" are novel alcohol/aldehyde dehydrogenases which the ordering person et al. identified the genes and cloned and sequenced from the strain of DSM 4025 deposited before Deutsche Sammlung von Mikroorganismen in Göttingen (Germany) as described in the patent application EP 96115001.8 which is a priority application of USSN 09/470,667.]

7. The sequencing primers for 5

Primer for Analysis 1

A697f: 5'- TACGAAGCCC GTTGGATGAC - 3' (GC 11/20)

A1000r: 5'- TCGGGTTGAT CGACTGCAGA - 3' (GC 11/20)

Primer for Analysis 2

A"479f: 5'- TATTCGACGT CGATCGCGGT - 3' (GC 11/20)

A"780r: 5'- AACTGCTGAG GTGCCGTAGT - 3' (GC 11/20)

8. Others

[1] Please confirm the contents of our Order letter (two copies in English), and after dating and signature please send one copy back to us.

[2] Please cultivate the strain DSM 4025, which we sent, on the cultivation medium attached.

[3] Please prepare the chromosomal DNA from the above strain and amplify the DNAs by using the primers identified in the above Analysis 1 and Analysis 2, respectively and

[4] Please prepare an experimental report (in Japanese) describing the experimental method, conditions and results in detail, which report should be executed by the person responsible to the experiment, as well as the sequencing of the respective PCR products as described in the above Analysis 1 and Analysis 2. Incidentally, we would kindly ask you to cooperate us in preparing a Declaration of your responsible person in this experiment later.

End

Declaration Y. Murata
Exhibit-5 (2/2)

Declaration Y. Murata
Exhibit -6

GENERAL PROTOCOLS

Roche's request to Sawady Technology Co. Ltd.

The flow of the actions that Nippon Roche Research Center (Roche) and Sawady Technology Co. LTD. (SAWADY) will take are as described below:

- Roche will send SAWADY the following two materials:

(1) one ampule of the strain DSM 4025 newly furnished from DSMZ; and

(2) two agar plates (NS2) to recover the strain DSM 4025;

with three kinds of documents:

(1) Order letter which includes description of the mutual understanding between Roche and SAWADY;

(2) The ORDER FORM; and

(3) The GENERAL PROTOCOLS (this paper) together with its translation.

- SAWADY will be involved in the following actions for the sequencing experiment which is ordered by Roche this time:

a. Copying the receipt of the strain DSM 4025 and papers attached thereto;

b. Cultivating the strain DSM 4025 preserved in an ampule on the agar plate (NS2) at 27°C for 3-5 days;

c. Preparing the chromosomal DNA from the culture obtained through the above b;

d. Synthesizing two pairs of the primers (A697f , A1000r , A"479f and A"780r) identified in the ORDER FORM;

e. Amplifying the target two regions by PCR with the respective pairs of the primers;

f. Performing direct sequencing of the PCR products, respectively;

g. Preparing an experiment report (which describes the precise protocols used to sequence the PCR products and the results); and

h. Sending (a) and (g) mentioned above to Roche.

End

Declaration & Minutes
Exhibit-7

[訳文]

ジェネラル プロトコール
サワデーテクノロジーに対するロシュの依頼事項

日本ロシュ研究所（Roche）及び株式会社サワディー・テクノロジー（SAWADY）が執り行う手続きの流れは、次の通りです。

- Roche は、次の２種類の材料：

（１）DSMZ から新たに分譲を受けた菌株 DSM 4025 のアンプル１本、及び

（２）菌株 DSM 4025 を起こすための２個の寒天平板培地(NS2)；を

次の３種類の文書；

（１）Roche 及び SAWADY の間の相互理解事項の記載を含む依頼状；

（２）依頼書；及び

（３）訳文が添付されたジェネラル プロトコール（この書面）

と共に SAWADY に送付します。

- SAWADY は今回 Roche が依頼する配列決定実験に際して次の手続きを含めてお執り進め願います：

ａ．菌株 DSM 4025 の受領書及び菌株に添付された書面の写し作成；

ｂ．アンプル中に保存された菌株 DSM 4025 を、寒天平板培地(NS2)上にて、２５℃で、３～５日間培養；

ｃ．上記ｂにて得られた培養物からの染色体ＤＮＡの調製；

ｄ．ORDER FORM に特定しました２対のプライマー(A697f , A1000r , A"479f 及び A"780r)の合成；

ｅ．それぞれのプライマー対を用いて PCR により２つの標的領域を増幅；

ｆ．それぞれの PCR 生成物の直接配列決定の実施；

ｇ．実験報告書（PCR 生成物の配列決定に使用した正確なプロトコール、及び結果を記述する）の作成；並びに

ｈ．上記(a)及び(g) の書類の Roche への送付。

以上

received
Aug. 27, 2002
M. Shinjoh

Sawady → → → KOLA
KOLA御中

注文書

サワディー管理番号 SW-002

お客様名 新城 様 平成 12年 8 月 17 日
所属 日本ロシュ研究所
住所 神奈川県鎌倉市梶原200
Tel Fax
Email

依頼内容:・菌体よりクロモゾームDNA抽出

納品状態:

サンプル: 菌体
DSM4025 (凍結乾燥品)
N2寒天培地 (2枚)
取り扱い説明書

Declaration K. Murata
Exhibit -8

備考:

その他、連絡事項

KOLA記入欄

予定納期 その他サワディーへの連絡事項等

内容をご確認いただき、ご不明の点はご連絡をいただけますようにお願いいたします。

(株)サワディーテクノロジー
〒171-0022 東京都豊島区南池袋2-9-9 第一池袋ホワイトビル1F
Tel: 03-3988-4633 Fax: 03-3982-5666
Email: product@sawady.com 担当/中川 温子

[T5] Order letter from Sawady to Kyurin dated Aug. 17, 2000

Four pages including this page.
Sawady to KOLA

To KOLA

ORDER SHEET

Sawady No. SW-002
Aug. 17, 2000

Client name: Dr. Shinjoh
Organization: Nippon Roche Research Center
Address: 200 Kajiwara Kamakura Kanagawa

Order: Extraction of chromosomal DNA from cells
Shipping form:
Sample: Cells
DSM4025 (Lyophilized)
N2** agar plates
Protocols
Others: Other information
KOLA memo: Planned delivery date Other information to Sawady

Please confirm the items and let us know if you have any questions.

K.K. Sawady Technology
171-0022 Dai-ichi Ikebukuro White Building 1F
2-9-9, Minami-Ikebukuro, Toshima-ku, Tokyo
Tel; 03-3988-4633 Fax; 03-3982-5666
Email:product@sawady.com Atsuko Nakagawa

Declaration Y. Muraoka
Exhibit-9

EX. F

Docket No: C38435/109700CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :)

Akira ASAKURA *et al.*) Examiner: M. Walicka

Serial No.: 09/470,667) Art Unit: 1652

Filed: December 22, 1999)

For: **NOVEL ALCOHOL/ALDEHYDE DEHYDROGENASES**)

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF MR. MASAO MASHITA UNDER 37 C.F.R. § 1.132

Sir:

I, Masao Mashita, a citizen and resident of Japan, hereby declare as follows:

1. I am employed by Sawady Technology Co., Ltd., 1-29-10, Maeno-cho, Itabashi-ku, Tokyo, Japan 174-0063 ("Sawady"). I hold the position of Sales & Marketing Director at Sawady. One of my duties at Sawady is to coordinate nucleic acid sequencing orders for clients of Sawady.

2. By way of background, Sawady is an independent commercial entity that is not affiliated with the Nippon Roche Research Center of Nippon Roche K.K. ("NRKK"). Among the services provided by Sawady to its clients is the sequencing of nucleic acid molecules.

It is in this capacity that I was initially contacted by Dr. Masako Shinjoh of NRKK regarding Sawady's ability and interest in sequencing a certain part of the chromosomal DNA derived from *Gluconobacter oxydans* DSM 4025 as set forth in more detail below.

3. As a follow up to my discussions with Dr. Shinjoh, on August 8, 2000, I was asked by Mr. Naoki Itoh, NRKK's Patent & Licensing, Manager, to have Sawady sequence certain portions of the chromosomal DNA of *Gluconobacter oxydans* DSM 4025 using two pairs of primers identified by NRKK. On the same day, Mr. Itoh forwarded to me via e-mail (i) an order letter, (2) an order form, and (3) general protocols to be used in the sequencing. A copy of Mr. Itoh's e-mail (and attachments) is attached as Exhibit 1 and an English translation of the e-mail is attached as Exhibit 2.

4. On August 11, 2000 I received an e-mail from Dr. Shinjoh (original written in Japanese attached as Exhibit 3; its English translation as Exhibit 4) indicating that she would send to me, via overnight courier, a package containing an ampoule of lyophilized cells of *Gluconobacter oxydans* DSM 4025 on August 16, 2000.

5. On the morning of August 17, 2000, I received the package referenced in Dr. Shinjoh's August 11, 2000 e-mail.

6. As is our standard practice, I engaged Mr. Yoshitaka Murata of K.K. Kyurin Corporation (27-25, Morishita-cho, Yahatanishi-ku, Kitakyushu-shi, Fukuoka-ken, 806-0046 Japan) ("Kyurin") to reconstitute the lyophilized cells in the ampoule I received from Dr. Shinjoh, to grow up those cells, and to isolate chromosomal DNA from those cells. Specifically, on August 17, 2000, I sent the ampoule I received from Dr. Shinjoh to Mr. Murata's company together with an instruction letter requesting that Mr. Murata provide me with isolated chromosomal DNA from the lyophilized cells in the ampoule. A copy of our order letter to Kyurin is attached as Exhibit 5 (in Japanese) and its English translation is attached as Exhibit 6.

7. On August 18, 2000, I received, confirmation that Mr. Murata received the ampoule and order letter.

8. On September 1, 2000, I received a package from Mr. Murata containing chromosomal DNA isolated from *Gluconobacter oxydans* DSM 4025 cells reconstituted from the lyophilized cells contained in the ampoule I sent to Mr. Murata. *See* the copy of the DECLARATION OF YOSHITAKA MURATA UNDER 37 C.F.R. §1.132 attached as Exhibit 7 (without Exhibits).

9. Using the isolated DNA forwarded from Mr. Murata, I supervised the nucleotide sequencing conducted by Mr. Susumu Yamashita at

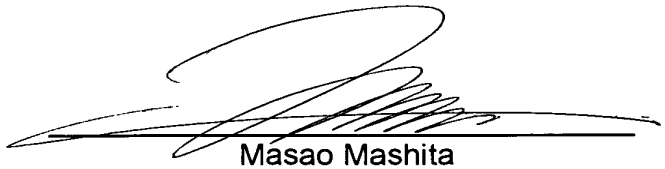
Sawady in accordance with the instructions of Dr. Shinjoh, the results of which are reported in the Experimental Report attached as Exhibit 8 (in Japanese) and its English translation as Exhibit 9.

10. The Experimental Report (non-finalized) was forwarded to Dr. Shinjoh on October 10, 2000 via Mr. Itoh. The Experimental Report was finalized and executed on September 12, 2002.

11. In sum, the ampoule containing the lyophilized *Gluconobacter oxydans* DSM 4025 cells that I received from Dr. Shinjoh on August 17, 2000 was the same ampoule that I forwarded to Mr. Murata at Kyurin on the same day I received it. And, the chromosomal DNA I received from Mr. Murata on September 1, 2000 was the same DNA that was used in the sequencing experiments performed under my supervision at Sawady, the results of which are reported in Exhibits 8 and 9.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 12. Sep 2002



Masao Mashita

Shinjoh, Masako [NRRC~Tokyo]

差出人: Itoh, Naoki [CPO~Tokyo]
送信日時: 2000年8月8日火曜日 午前 11:50
宛先: 'sales@sawady.com'
CC: Shinjoh, Masako [NRRC~Tokyo]
件名: 遺伝子配列決定依頼の件

（株）サワディー・テクノロジー
営業担当取締役
間 下 正 雄 様

お世話になっております。
先日は、ご多忙のところ打合わせのお時間を戴きまして有難うございました。
打合わせにおいて申し上げました以下の書面をお送りしますので、内容ご確認ください。
(1) Order Letter
(2) ORDER FORM（日本語の正本、及びその英訳）
(3) GENERAL PROTOCOLS（英文の正本、及びその日本語訳）

試料を、打合わせに従いまして８月１６日に発送いたします（１７日御社着）ので、上記書面に訂正の必要がありましたら至急にお知らせください。

以上、宜しくお願い申し上げます。

伊藤直樹
日本ロシュ（株）
ライセンシング＆パテントグループ


SAWADY-1.doc


SW-ORDERFORM-1.doc


SW-ORDERFORM-JP.doc


SW-GEN-PRO.doc


SW-GEN-PRO-JP.doc

Declaration MMashita
Exhibit-1

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[T2] E-mail from Mr. Itoh to Mr. Mashita dated Aug. 8, 2000

From: Itoh, Naoki

Date: August 8, 2000 11:50 am

To: Sales@sawady.com

cc: Shinjoh, Masako {NRRC~Tokyo}

Subject: Determination of nucleotide sequence

K. K. Sawady Technology
Sales Marketing Director
Mr. Masako Mashita,

Thank you for your usual service and taking a time for us in your busy schedule the other day.
Please confirm the following papers I explained at that time
(1) Order Letter
(2) ORDER FORM (Original in Japanese, Translation in English)
(3) GENERAL PROTOCOLS ((Original in English, Translation in Japanese)
According to our discussion, we will send the sample on Aug. 16 (it will arrive at your company on Aug. 17). If you have any amendment for the papers above, let us know as soon as possible.

Best regards,
Naoki Itoh
Nippon Roche K.K.
Licensing&Patent group

Attached files:

<SAWADY-A.doc>; <SW-ORDERFORM-1.doc>; <SW-ORDERFORM-JP.doc>
<SW-GEN-PRO.doc>; <SW-GEN-PRO-JP.doc>

Declaration M Mashita
Exhibit -2

3

Shinjoh, Masako {NRRC~Tokyo}

差出人: Shinjoh, Masako {NRRC~Tokyo}
送信日時: 2000年8月11日金曜日 午後 3:53
宛先: 'Sales@sawady.com'
CC: Itoh, Naoki {CPO~Tokyo}
件名: PCR-sequencing 依頼

間下さん

いつもお世話になります。
今回の、sequencingもよろしくお願いいたします。
注文の詳細は、弊社伊藤がお伝えしましたように、来週水曜日、8月16日に材料とともにクロネコ宅急便で、17日午前到着指定でおくります。

今回は、支払に係る、こちらの注文番号をお伝えいたします。
注文番号: W0005031

それでは、解析の方、よろしくお願いいたします。

新城雅子

日本ロシュ研究所
所属:応用微生物部
氏名:新城雅子
住所: 鎌倉市梶原200
TEL: 0467-47-2226
PAX:0467-45-6812

Declaration M. Mashita
Exhibit -3

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[T3] E-mail from Dr. Shinjoh to Mr. Mashita dated Aug. 11, 2000

From: Shinjoh, Masako

Date: August 11, 2000 3:53 pm

To: Sales@sawady.com

cc: Itoh, Naoki {CPO~Tokyo}

Subject: PCR-sequencing order

Dear Mr. Mashita,

Thank you for your usual service.

I'd like to ask you this sequencing request.

The details of this order and our sample will be sent next Wednesday, Aug. 16, as Mr. Itoh has already let you know via KORONEKO-TAKKYUBIN to arrive to your site in the morning of Aug. 17.

I inform our order number relating the payment;

order number: W0005031.

Could you please perform the analysis.

Best regards,

Masako Shinjoh

NRRC, Applied Microbiology, Masako Shinjoh,

TEL: 0467047-2226 FAX; 0467-45-6812

Declaration M. Mashita
Exhibit-4

received
Aug. 27, 2002
M. Shinjoh

Sawady → → → KOLA
KOLA御中

注文書

サワディー管理番号 SW-002

お客様名　新城　様　　平成 12年　8 月　17 日
所属　日本ロシュ研究所
住所　神奈川県鎌倉市梶原200
Tel　　Fax
Email

依頼内容:・菌体よりクロモゾームDNA抽出

納品状態:

Declaration MMashita
Exhibit-5

サンプル: 菌体
DSM4025（凍結乾燥品）
N2寒天培地（2枚）
取り扱い説明書

備考:

その他、連絡事項

KOLA記入欄

予定納期 その他サワディーへの連絡事項等

内容をご確認いただき、ご不明の点はご連絡をいただけますようにお願いいたします。

(株)サワディーテクノロジー
〒171－0022 東京都豊島区南池袋2－9－9 第一池袋ホワイトビル1F
Tel: 03－3988－4633 Fax: 03－3982－5666
Email: product@sawady.com 担当／中川 温子

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[T5] Order letter from Sawady to Kyurin dated Aug. 17, 2000

Four pages including this page.
Sawady to KOLA

To KOLA

ORDER SHEET

Sawady No. SW-002
Aug. 17, 2000

Client name: Dr. Shinjoh
Organization: Nippon Roche Research Center
Address: 200 Kajiwara Kamakura Kanagawa

Order: Extraction of chromosomal DNA from cells
Shipping form:
Sample: Cells
DSM4025 (Lyophilized)
N2** agar plates
Protocols
Others: Other information
KOLA memo: Planned delivery date Other information to Sawady

Please confirm the items and let us know if you have any questions.

K.K. Sawady Technology
171-0022 Dai-ichi Ikebukuro White Building 1F
2-9-9, Minami-Ikebukuro, Toshima-ku, Tokyo
Tel; 03-3988-4633 Fax; 03-3982-5666
Email:product@sawady.com Atsuko Nakagawa

Declaration
M. Mashita
Exhibit-6

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Docket No: C38435/109700CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :	)	
Akira ASAKURA *et al.*	)	Examiner: M. Walicka
Serial No.: 09/470,667	)	Art Unit: 1652
Filed: December 22, 1999	)	
For: **NOVEL ALCOHOL/ALDEHYDE DEHYDROGENASES**	)	

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF MR. YOSHITAKA MURATA UNDER 37 C.F.R. § 1.132

Sir:

I, Yoshitaka Murata, a citizen and resident of Japan, hereby declare as follows:

1. I am employed by K.K. Kyurin Corporation, 27-25, Morishita-cho, Yahatanishi-ku, Kitakyushu-shi, Fukuoka-ken, 806-0046 Japan ("Kyurin"). I hold the position of Scientist (Kyurin Omtest Laboratory Dept. "KOLA") at Kyurin. One of my duties at Kyurin is to coordinate the preparation of chromosomal DNA from various cell lines in response to orders from clients of Kyurin. A copy of my *curriculum vitae* is attached as Exhibit 1.

2. By way of background, Kyurin is an independent commercial entity that is not affiliated with the Nippon Roche Research Center of

Nippon Roche K.K. ("NRKK"). Among the services provided by Kyurin to its clients is the preparation of chromosomal DNA from various kinds of cell lines. It is in this capacity that I was contacted by Mr. Masao Mashita of Sawady Technology Co., Ltd., 1-29-10, Maeno-cho, Itabashi-ku, Tokyo, Japan 174-0063 ("Sawady") regarding our ability and interest in preparing chromosomal DNA by reconstituting and growing up a lyophilized sample of *Gluconobacter oxydans* DSM 4025 as set forth in more detail below.

3. At the beginning of August, 2000, I was asked by Mr. Mashita to have Kyurin reconstitute, grow up, and harvest chromosomal DNA from a lyophilized sample of *Gluconobacter oxydans* DSM 4025 cells that he would provide to me.

4. On August 10, Mr. Mashita sent a letter to Kyurin via facsimile (a copy of the original facsimile in Japanese is attached as Exhibit 2 and its translation in English is attached as Exhibit 3). This letter confirmed our agreement with Sawady that Kyurin would conduct the requested work and included an "ORDER FORM" (original written in Japanese, a copy of which is attached as Exhibit 4; its English translation is attached as Exhibit 5) and a set of "GENERAL PROTOCOLS" (original written in English, Exhibit 6; its Japanese translation as Exhibit 7) describing the methods to be used by us for isolating the requested chromosomal DNA.

5. On August 18, 2000, I received a package from Mr. Mashita via overnight courier. The package contained an ampoule identified as containing lyophilized cells of *Gluconobacter oxydans* DSM 4025 and an order sheet from Sawady (original written in Japanese, a copy of which is attached as Exhibit 8; its English translation is attached as Exhibit 9).

6. As soon as I received the package, I stored the package in a refrigerator accessible only to authorized Kyurin personnel at 4°C. Later that day, Dr. Sugama, Director, KOLA Kyurin, at my direction, sent an e-mail to Mr. Mashita to confirm receipt of the ampoule and the order letter.

7. On August 26, 2000, I gave Ms. Masako Nomaguchi, a researcher employed by Kyurin, the ampoule I received from Mr. Mashita on August 18, 2000, identified as containing lyophilized cells of *Gluconobacter oxydans* DSM 4025, and instructed Ms. Nomaguchi to reconstitute the lyophilized cells contained in the ampoule, to grow up those cells, and to isolate chromosomal DNA from those cells.

8. On August 31, 2000, Ms. Nomaguchi informed me that she had completed isolating the chromosomal DNA from the cells grown up from the *Gluconobacter oxydans* DSM 4025 sample I had given her, which I had received from Mr. Mashita on August 18, 2000. Ms.

Nomaguchi collected the isolated chromosomal DNA in a 1.5ml tube, which was labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831."

9. On August 31, 2000, I placed the 1.5ml tube labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831" containing the isolated chromosomal DNA prepared by Ms. Nomaguchi into a shipping package. That same day, I forwarded to Mr. Mashita the package containing the tube labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831" containing the chromosomal DNA isolated from the *Gluconobacter oxydans* DSM 4025 cells prepared by Ms. Nomaguchi.

10. In sum, the ampoule containing the lyophilized *Gluconobacter oxydans* DSM 4025 cells that I received from Mr. Mashita on August 18, 2000 was the same ampoule that I gave to Ms. Nomaguchi, who reconstituted the cells, grew them up, and isolated genomic DNA from them. And, the chromosomal DNA that Ms. Nomaguchi isolated from the reconstituted *Gluconobacter oxydans* DSM 4025 cells was the same DNA contained in the tube labeled "SW-2 / DNeasy 28ng / μl 000831 * SW-2 / Sepagene 0.508 μg / μl 000831" that I forwarded to Mr. Mashita on August 31, 2000.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated:_____

_____
Yoshitaka Murata

試験報告書

試験番号 F－903

試験表題：菌株 DSM4025 における遺伝子配列の決定

平成 14 年 9 月 12 日

お客様名：日本ロシュ研究所　日本ロシュ株式会社

神奈川県鎌倉市梶原 200（〒247－8530）

株式会社 サワディー・テクノロジー
東京都板橋区前野町 1 丁目 29 番 10 号（〒174－0063）

試験報告書

表題：

菌株 DSM4025 の遺伝子配列の決定

試験番号：

F-903 （SW-002）

試験委託者

名称：日本ロシュ研究所 日本ロシュ株式会社

所在地：神奈川県鎌倉市梶原 200 （〒247-8530）

試験実施者：

名称：株式会社 サワディー・テクノロジー

所在地：東京都板橋区前野町 1 丁目 29 番 10 号 （〒174-0063）

運営管理者：増尾 正則

試験責任者

氏名：間下 正雄

所属：株式会社 サワディー・テクノロジー

氏名：村田 義隆

所属：株式会社 キューリン

試験担当責任者

氏名：山下 進

所属：株式会社 サワディー・テクノロジー

氏名：野間口 雅子

所属：株式会社 キューリン

実施期間

試験開始日：平成 12 年 8 月 26 日
試験終了日：平成 12 年 9 月 5 日
最終報告書提出日：平成 14 年 9 月 12 日

試料

凍結乾燥菌株
供給源：日本ロシュ研究所 日本ロシュ株式会社

使用試薬および使用機器

この試験を実施するにあたって使用した試薬および機器の一覧は Attachment（A）のとおりである。

方法：

1. 提供された菌株 DSM4025 の育成

菌株に添付されていた菌株育成のプロトコールに従いアンプルを処理し凍結乾燥品を滅菌済みのハサミとピンセットを使い半分にした。
半分をアンプルに戻し、半分をその後の実験に用いた。
菌体を懸濁する際には Medium ではなく ddH_2O 200 μl を加えた。
菌体が解けにくかったため、同様に ddH_2O を更に 200 μl を加えた。
200 μl を NS2 培地に塗布した。
27℃で、4 日間培養を行った。

白金耳でプレート半分部分をなぞるように鈎菌し、dH_2O に溶かし込んだ。
A_{550} を測定。5 倍希釈で 0.3061 になるまで徐々に菌を希釈した。
12,000rpm、5 分にて菌体を回収した。

2. 染色体 DNA の調製

SepaGene（三光純薬株式会社）、DNeasy Tissue Kit（株式会社キアゲン）の 2 種類のキットを使用して染色体 DNA の調製を行った。
両方法ともキットに添付されているプロトコールに従って行った。
SepaGene は抽出法 I の手順にて行った。

SepaGene（抽出法Ⅰ）を使用したゲノムDNA抽出

1. A_{550}の測定値5倍希釈で0.3061となったものを　12,000rpm、5分の遠心を行い、集菌した。
2. 上清を除去した後、Tris-HCl（pH8.0）100uLを加え、均一に懸濁させた。
3. 室温で10分間静置した。
4. この懸濁液にチオシアン酸グアニジン 100uLを加え、ピペットで緩やかに混和した。
5. クロロホルム 50%を含む吸着剤 700uL と、酢酸ナトリウム溶液400uLを加えた。
6. マイクロチューブのふたを閉め、乳濁化するまで10秒間上下に激しく振盪混和した。
7. 12,000rpm、15分間遠心した。
8. 核酸を含む上層を別のマイクロチューブに分取した。
9. 酢酸緩衝液を55uL加えた。
10. 605uLのイソプロピルアルコールを加え、軽く転倒混和した。
11. 12,000rpm、15分間遠心した。
12. 上清を静かに除去し、70%エタノール1mLを加え軽く転倒混和した。
13. 12,000rpm、15分間遠心した。
14. 上清を静かに除去し、核酸ペレットを軽く乾燥させた。

DNaseyを使用したゲノムDNA抽出

1. A_{550}の測定値5倍希釈で0.3061となったものを　12,000rpm、5分の遠心を行い、集菌した。
2. 180uLのATL　Bufferを加え懸濁した。
3. 20uLの Proteinase Kを加え、混和し、細胞が完全に溶解するまで55℃でover　nightでインキュベートした。
4. 15秒間ボルテックスをかけた後、200uLのBuffer ALを加えよく混和した後、70℃で10分間インキュベートした。
5. 96－100%エタノールを200uL加え、十分に混和した。
6. 2ml のチューブにセットした DNeasy カラムに 5 を静かにのせ、8000rpmで1分遠心を行い、ろ液を除去した。
7. DNeasy カラムを新たな 2ml チューブにセットし、500uL の AW1 Bufferを加え、8000rpmで1分遠心し、ろ液を除去した。
8. DNeasy カラムを新たな 2ml チューブにセットし、500uL の AW2

Buffer を加え 15,000rpm で 3 分間遠心し、DNeasy メンブレンを乾燥させ、ろ液を除去した。

9. DNeasy カラムを 2ml チューブにセットし、200uL の Buffer AE（10mM Tris-HCl, pH9.0. 0.5mM EDTA, pH9.0）を加えた後、室温で 1 分間静置した後、8000rpm で 1 分間遠心し、溶出させた。
10. ステップ 9 をもう一度繰り返した。

3. プライマー作成

サワディー・テクノロジーにて指示されたプライマーを作成した。
合成後、オリゴパックカラム（OPC）にて精製を行った。

解析 1 のプライマー

Forward: A697f	5'－ TACgAAgCCC gTTggATgAC — 3'
Reverse: A1000r	5'－ TCgggTTgAT CgACTgCAgA — 3'

解析 2 のプライマー

Forward: A"479f	5' － TATTCgACgT CgATCgCggT － 3'
Reverse: A"780r	5' － AACTgCTgAg gTgCCgTAgT － 3'

4. 目的領域の PCR

方法 3 で合成を行った 2 ペアのプライマーを使用し、PCR を行い、目的領域の増幅を行った。

Let's Go PCR	48.5μL
primer	0.5μL (20μM)
DNA	1 μL
	50 μL

90℃	1min	
↓	以下 35cycle 繰り返す	
95℃	30 sec	}
50℃	30 sec	}
68℃	30 sec	}
↓		
72℃	30 min	
4℃	ストック	

電気泳動にて増幅領域を確認後、目的領域を切り出し、High pure PCR Product Purification Kit（ロシュダイアグノーシス）にて精製を行った。

5. 塩基配列決定

方法4で増幅したPCR産物をABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit（Applied Biosystems Japan）のプロトコールに従い解析を行った。

Terminator Ready Reaction Mix	8.0μL
Template	50 ng
primer	4 pmol
DW	
total	20μL

PCR条件は以下の通り。

25cycle 繰り返す		
96℃	10sec	}
50℃	5sec	}
60℃	4min	}

ABI Prism 377にセットし各サンプルを3μLアプライした。
overnightにて泳動を行った。

結果：

1. 染色体 DNA の調製

DNeasy で調製した DNA はステップ 10 のように、Buffer AE（10mM Tris-HCl, pH9.0, 0.5mM EDTA, pH9.0）100 μL に溶解した。
SepaGene で調製した DNA は TE Buffer（10mM Tris-HCl, pH8.0. 1mM EDTA, pH8.0） 50 μL に溶解し濃度を測定した。

```
DU530 S/N: 9706U3000073 1.03
31-AUG-00  10:30:36  NUCLEIC ACID  Double Ratio &Conc  Group 0315
PATHcm: 1.0000
```

Sample	Net A 260.0	Net A 280.0	Net A 230.0	260.0/ 280.0	260.0/ 230.0	
DNeasy 0001 ×20	0.030	0.015	0.014	2.041	2.180	DNeasy
DNeasy 0002 ×20	0.026	0.014	0.003	1.811	8.151	28ng/ul × 100ul
Sepa 0003 ×50	0.209	0.105	0.096	1.996	2.170	Sepagene
Sepa 0004 ×50	0.197	0.099	0.092	1.992	2.151	0.508ng/ul ×50ul

2. PCR

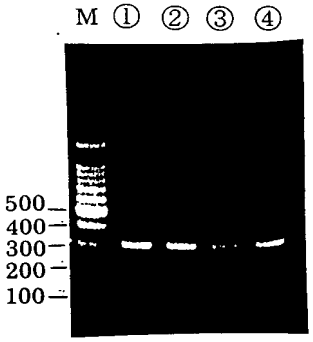
解析 1. 名称 Enzyme A

304bp

解析 2. 名称 Enzyme A"

302bp

電気泳動写真より目的の大きさの PCR 産物が得られている事が分かる。
2%アガロースゲル、PCR 産物は各 3 μL を泳動した。



① EnzymeA(nt697-1000)
② EnzymeA(nt697-1000)
③ EnzymeA(nt479-780)
④ EnzymeA(nt479-780)

①③は template DNA に SW-2/DNeasy を使用
②④た template DNA に SW-2/Sepagene を使用

3. 塩基配列決定
   指示通り、テンプレートに DNeasy にて抽出したゲノム DNA をもちいて、F／R のプライマーを用い両方向からシークエンス反応を行った。
   シークエンス配列は Attachment　B の通り
   波形データは　Attachment　C の通り

私たちは各々、この試験報告書が、株式会社キューリンにより私達に供給された *Gluconobacter oxydans* DSM 4025 の染色体 DNA から単離された増幅産物のクローニングと配列決定　（ Attachment B に示す ）についての真実で正確な記述であることを断言し、署名いたします。

日付 12. Sep. 2002　　株式会社　サワディー・テクノロジー

間下 正雄、営業部兼マーケティング部マネージャー

日付 13/Sep/2002

増尾 正則、運営管理者

Attachment (A)

使用試薬および使用機器

使用試薬

- NS2 培地（依頼者より提供）
- 核酸抽出剤 SepaGene （三光純薬株式会社）
- DNeasy Tissue Kit（株式会社 キアゲン）
- ABI Prism BigDye Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems Japan)
- AmpliTaq DNA Polymerase (Applied Biosystems Japan)
- Let's Go PCR Kit （株式会社 サワディー・テクノロジー）
- High Pure PCR Purification Kit （ロシュダイアグノーシス）

使用機器

- サンヨーインキュベーター MIR153 （サンヨー）
- ABI prism 377 (Applied Biosystems Japan)
- GeneAmp PCR System 9600 (Applied Biosystems Japan)
- ミニサイクラー （MJ Reserch）
- MicroCen13D （Herolab）
- Mupid ミニゲル泳動槽 （Mupid）

Attachment (B)

Sequences of the amplified products.

39F903 (697-1000)/A697f.Seq

TTNCGTGCCT GGGGCCAGAT CACCTATGAC CCCGTCACCA ACCTTGTCCA
CTACGGCTCG ACCGCTGTGG GTCCGGCGTC GGAAACCCAA CGCGGCACCC
CGGGCGGCAC GCTGTACGGC ACGAACACCC GTTTCGCCGT GCGTCCTGAC
ACGGGCGAGA TTGTCTGGCG TCACCAGACC CTGCCCCGCG ACAACTGGGA
CCAGGAATGC ACGTTCGAGA TGATGGTCAC CAATGTGGAT GTCCAACCCT
CGACCGAGAT GGAAGGTCTG CAGTCGATCA ANCGAAANNN NNNNNNNNNN
NNNNN

41F903 (697-1000)/A1000r.Seq

TTCCTCTTGG TCGAGGGTTG GACATCCACA TTGGTGACCA TCATCTCGAA
CGTGCATTCC TGGTCCCAGT TGTCGCGGGG CAGGGTCTGG TGACGCCAGA
CAATCTCGCC CGTGTCAGGA CGCACGGCGA AACGGGTGTT CGTGCCGTAC
AGCGTGCCGC CCGGGGTGCC GCGTTGGGTT TCCGACGCCG GACCCACAGC
GGTCGAGCCG TAGTGGACAA GGTTGGTGAC GGGGTCATAG GTGATCTGGC
CCCAGGCACC GGTCATCCAA CGGGCTTTGT AANNNNNNNN NNNNNNNNNN
N

43F903 (479-780)/A479f.Seq

AAAGCACTTT ATGGNCTCGA ACTCTCCGGC CCGATTGTCG CCAATGGCGT
CATCGTTGCG GGCTCGACCT GTCAGTATTC GCCGTTCGGC TGTTTCGTTT
CGGGCCACGA CTCGGCCACC GGTGAAGAGC TGTGGCGCAA CACCTTTATC
CCGCGCGCCG GCGAAGAGGG TGATGAGACC TGGGGCAATG ATTACGAGGC
CCGCTGGATG ACCGGCGTTT GGGGCCAGAT CACCTATGAC CCCGTTGGCG
GCCTTGTCCA CTACGGCACC TCAAGAGTTA ANANNNNNNN NNNNNNNNN

45F903 (479-780)/A780r.Seq

GACAAGGCTN NCACGGNGTC ATAGGTGATN TGGCCCCAAA CGCCGGTCAT
CCAGCGGGCC TCGTAATCAT TGCCCCAGGT CTCATCACCC TCTTCGCCGG
CGCGCGGGAT AAAGGTGTTG CGCCACAGCT CTTCACCGGT GGCCGAGTCG
TGGCCCGAAA CGAAACAGCC GAACGGCGAA TACTGACAGG TCGAGCCCGC
AACGATGACG CCATTGGCGA CAATCGGGCC GGACGAGTTC GAGACCATAT
CCGTGCCTTG ACCGCGATCG ACGTCCATAA ANNNNNNNNN NNNNNNNNNN

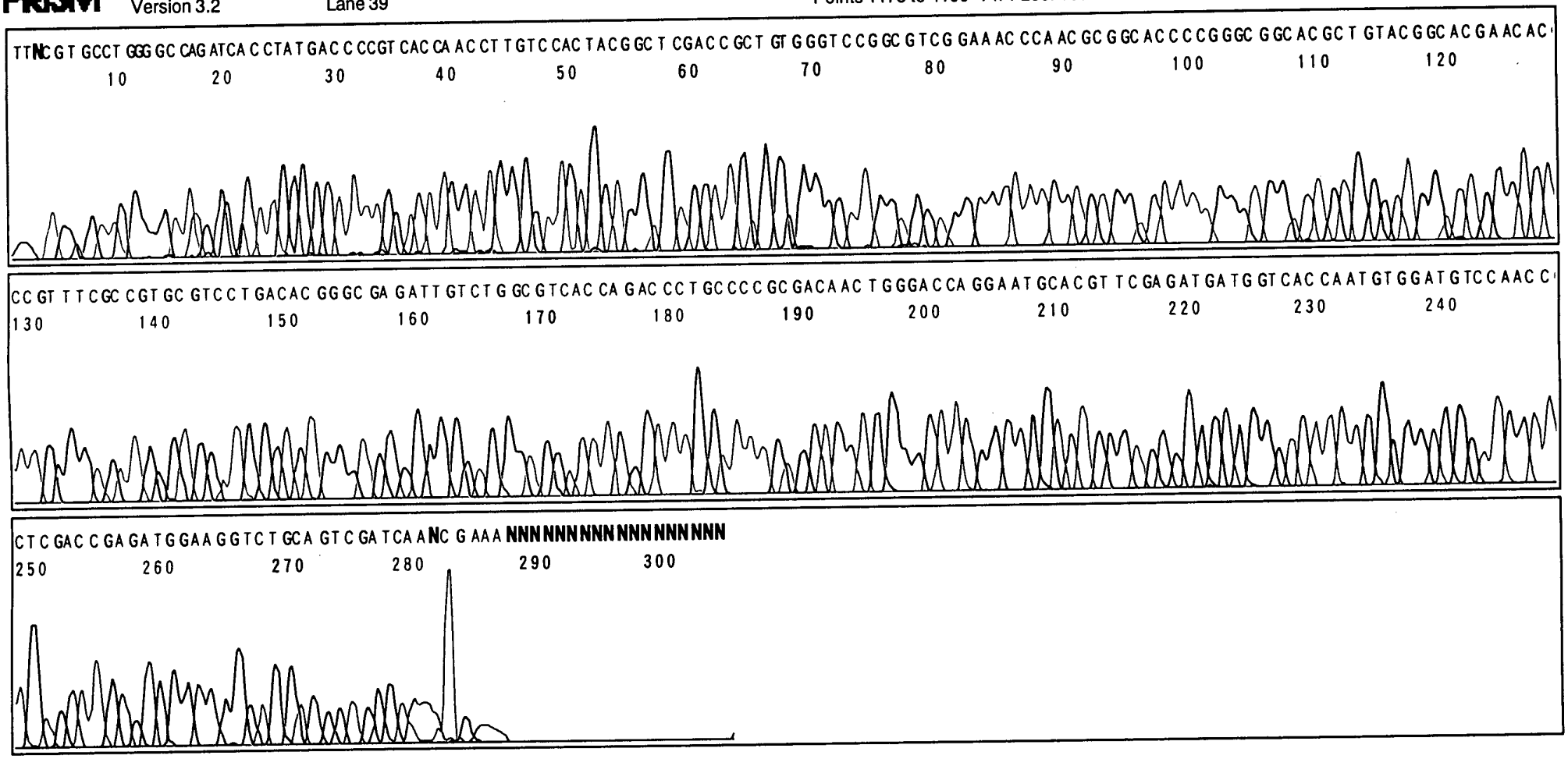
Model 377
Version 3.3
ABI100
Version 3.2

39•F903 (697-1000)/A697f

F903 (697-1000)/A697f
Lane 39

Signal G:1061 A:1044 T:410 C:700
DT{BD Set Any-Primer}
dRhodamine Teminator Matrix
Points 1175 to 4100 Pk 1 Loc: 1054

Tue, Sep 5, 2000 2:02 PM
Mon, Sep 4, 2000 6:18 PM
Spacing: 10.14{10.14}



Attachment C-1

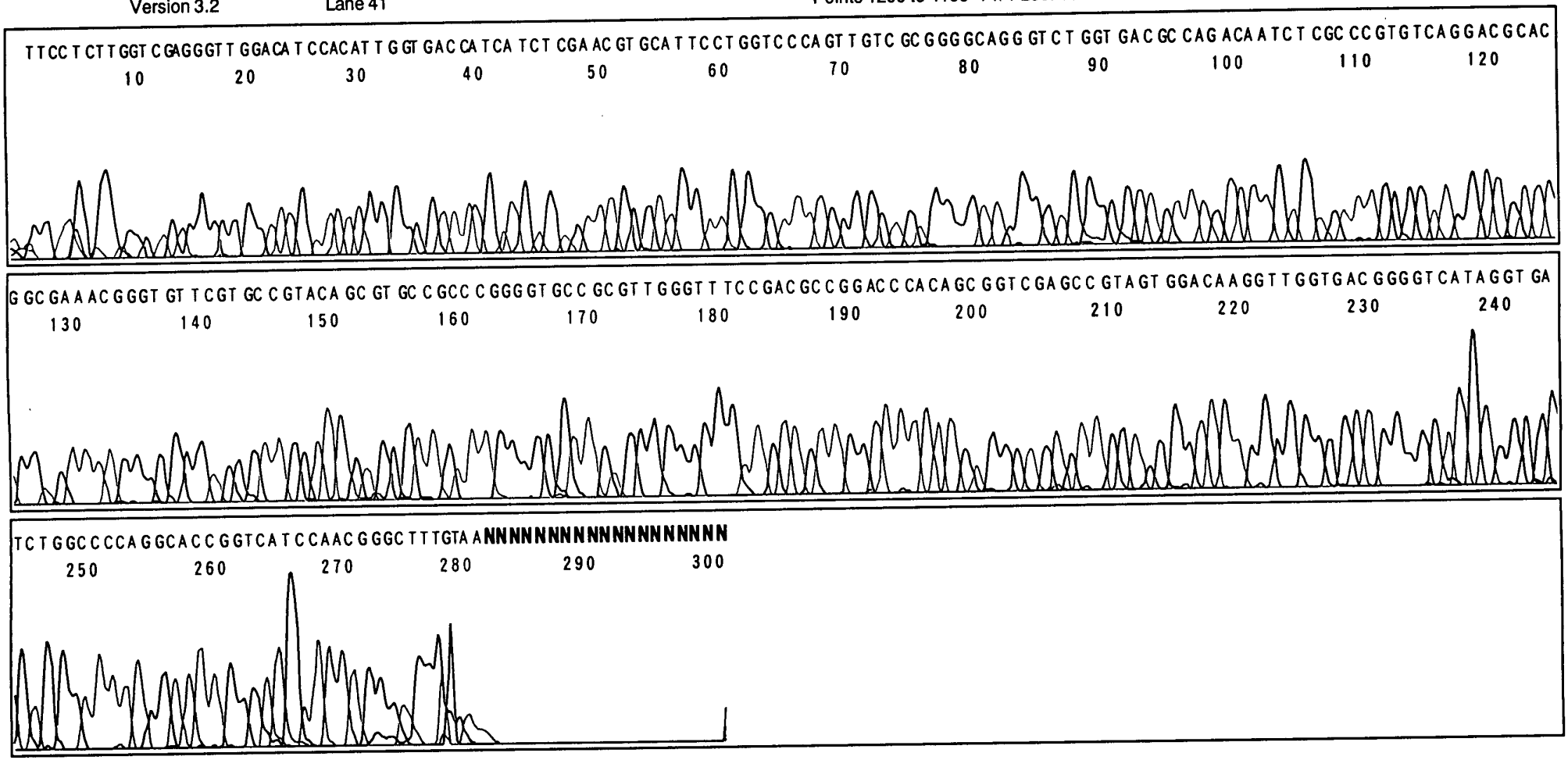
Model 377
Version 3.3
ABI100
Version 3.2

41•F903 (697-1000)/A1000r

F903 (697-1000)/A1000r
Lane 41

Signal G:1010 A:770 T:388 C:568
DT{BD Set Any-Primer}
dRhodamine Teminator Matrix
Points 1200 to 4100 Pk 1 Loc: 1053

Tue, Sep 5, 2000 2:03 PM
Mon, Sep 4, 2000 6:18 PM
Spacing: 10.05{10.05}



ABI PRISM

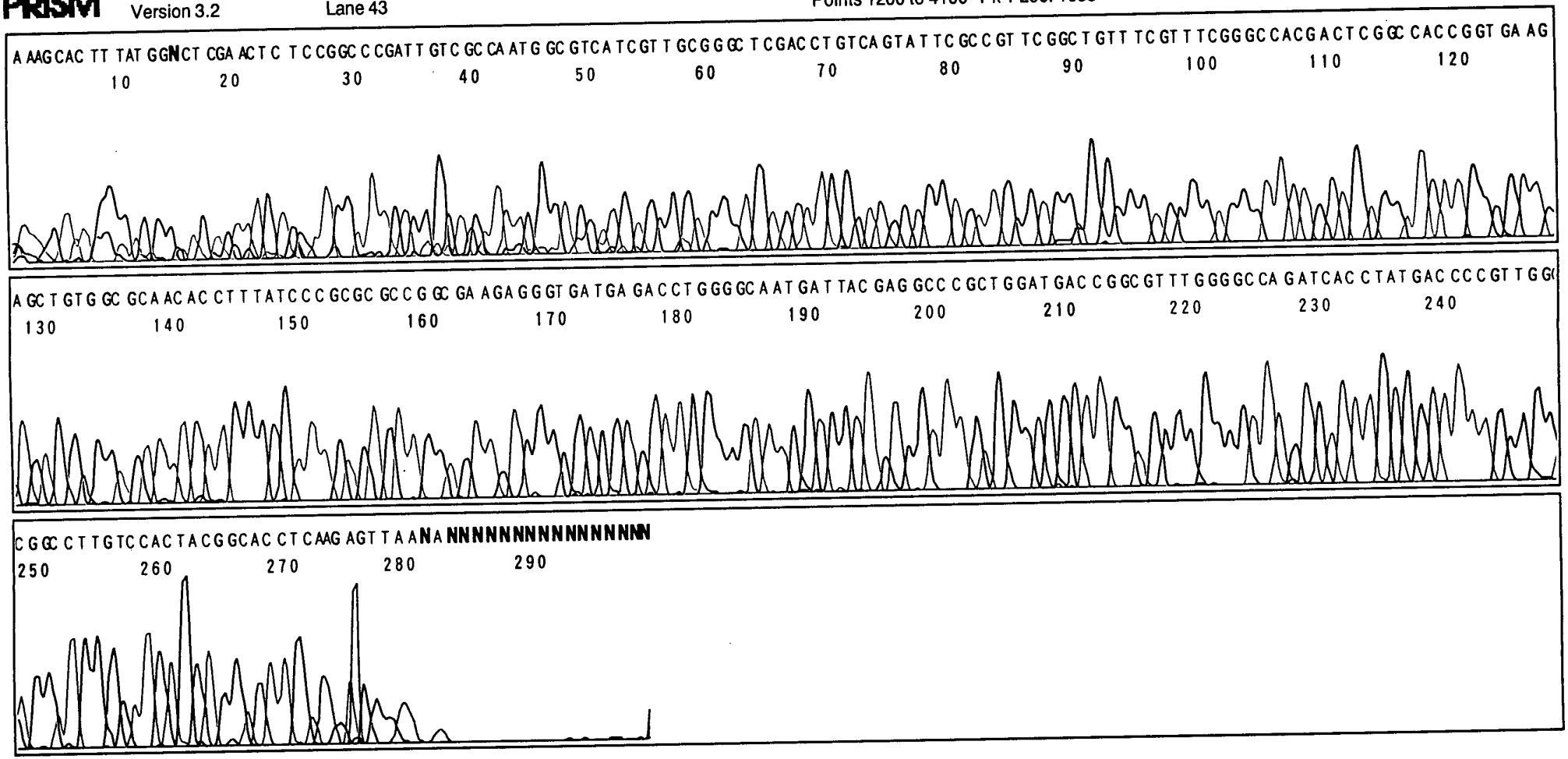
Model 377
Version 3.3
ABI100
Version 3.2

43•F903 (479-780)/A479f

F903 (479-780)/A479f
Lane 43

Signal G:523 A:379 T:211 C:222
DT{BD Set Any-Primer}
dRhodamine Teminator Matrix
Points 1200 to 4100 Pk 1 Loc: 1053

Tue, Sep 5, 2000 2:04 PM
Mon, Sep 4, 2000 6:18 PM
Spacing: 10.26{10.26}



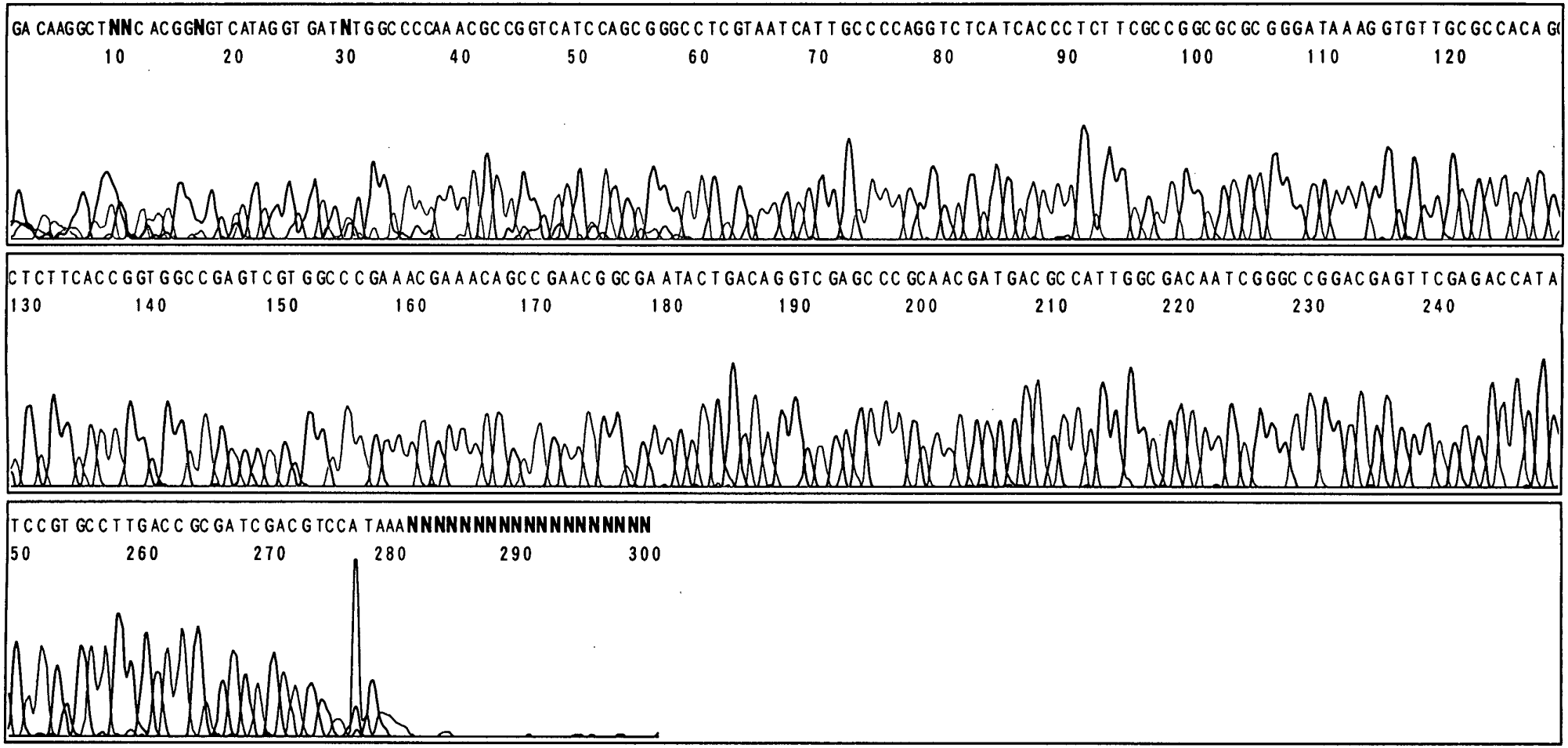
Attachment C-3

Model 377
Version 3.3
ABI100
Version 3.2

45•F903 (479-780)/A780r
F903 (479-780)/A780r
Lane 45

Signal G:658 A:610 T:254 C:336
DT{BD Set Any-Primer}
dRhodamine Teminator Matrix
Points 1200 to 4100 Pk 1 Loc: 1053

Tue, Sep 5, 2000 2:04 PM
Mon, Sep 4, 2000 6:18 PM
Spacing: 10.22{10.22}



(Translation)

Experimental Report

Experiment Number: F-903

Theme: Sequencing of the genes of the strain DSM 4025

Date: September 12, 2002

Name of Client:

Nippon Roche Research Center, Nippon Roche K.K. (NRKK)
Kajiwara 200, Kamakura-shi, Kanagawa-ken, 247-8530, Japan

K.K. Sawady Technology
1-29-10, Maeno-cho, Itabashi-ku, Tokyo, 174-0063, Japan

Experimental Report

Theme: Sequencing of the genes of the strain DSM 4025

Experiment Number: F-903 (SW-002)

Requester of the Experiment:

Nippon Roche Research Center, Nippon Roche K.K.
Kajiwara 200, Kamakura-shi, Kanagawa-ken, 247-8530, Japan

Experimenter (company):

K.K. Sawady Technology
1-29-10, Maeno-cho, Itabashi-ku, Tokyo, 174-0063, Japan
Name of COO: Masanori Masuo

Person responsible for the Experiment:

Name: Masao Mashita
Company: K.K. Sawady Technology
Name: Yoshitaka Murata
Company: K.K. Kyurin

Persons responsible for performing the Experiment:

Name: Susumu Yamashita
Company: K.K. Sawady Technology

Name: Masako Nomaguchi
Company: K.K. Kyurin

Term of the Experiment:

Initiated on: August 2000
Finished on: September 2000
Date of final report: September 12, 2002

Sample:

Lyophilized strain in an ampoule
Supplied by Nippon Roche Research Center, Nippon Roche K.K.

Reagents and Devices:

The reagents and the instruments used for performing the present Experiment are listed in Attachment (A).

Methodology:

1. Cultivation of the strain DSM 4025 supplied:

The ampoule was treated in accordance with the protocol for the cultivation of the strain attached to the ampoule, wherein the lyophilized material was cut into two equal pieces with a sterilized pair of scissors and pincette. One piece was returned to the ampoule and the other was used for the experiment.

For suspending the cells, 200 µl of ddH_2O was added instead of a medium. As the strain was hard to be suspended, an additional 200 µl of ddH_2O was added. 200 µl of the suspension was added to NS2 medium. The medium was incubated at 27°C for 4 days.

Cells from a half of the plate were scraped with a platinum loop (to collect the strain) to be dissolved in dH_2O. The cell suspension was gradually diluted to A_{550} of 0.3061 when diluted by 5 fold. The cells of the strain were collected by centrifugation at 12,000 rpm for 5 minutes.

2. Preparation of chromosomal DNA

The chromosomal DNA was prepared using two commercial kits, which are SepaGene (Sankou Junyaku K.K.) and DNeasy Tissue Kit (K.K. Qiagen). Both the kits were used in accordance with the protocols attached to the kits. With respect to SepaGene, Extraction Method I was used.

Extraction of genome DNA with SepaGene (Extraction Method I)

1. Collected the cells from the cell suspension of A550 = 0.3061 obtained by 5-fold dilution of above mentioned cell suspension by centrifugation at 12,000 rpm for 5 minutes.
2. After discarding the supernatant, the cells were suspended homogeneously by adding 100 µl of Tris-HCl (pH8.0).

3. The cells were maintained at room temperature for 10 minutes.
4. Added 100 µl of guanidine-thiocyanate into the cell suspension and mixed the solution gently with a pipette.
5. Added the 700 µl absorbant containing 50% chloroform and 400 µl of sodium acetate.
6. Closed the tube and mixed vigorously until the solution became emulsion for 10 seconds.
7. Centrifuged at 12,000 rpm for 15 minutes.
8. Took the upper phase containing nucleic acids.
9. Added 55 µl of acetate buffer.
10. Added 605 µl of isopropyl alcohol and mixed gently.
11. Centrifuged at 12,000 rpm for 15 minutes.
12. Discarded the supernatant, added 1 ml of 70% ethanol and mixed gently.
13. Centrifuged at 12,000 rpm for 15 minutes.
14. Discarded the supernatant and dried the nucleic acid pellet briefly.

Extraction of genome DNA with DNeasy (Extraction Method II)

1. Collected the cells from the cell suspension of A550 = 0.3061 obtained by 5-fold dilution of the above-mentioned cell suspension by centrifugation at 12,000 rpm for 5 minutes.
2. Added 180 µl ATL Buffer and suspended the cells.
3. Added 20 µl of Proteinase K, mixed the suspension and incubated at 55°C for 1 overnight until the cells were completely lysed.
4. Vortexed for 15 seconds, added 200 µl AL Buffer, mixed well, and incubated the suspension at 70°C for 10 minutes.
5. Added 96 to 100% ethanol and mixed well.
6. Loaded the solution obtained in step 5 gently onto a DNeasy column set on a 2 ml tube, centrifuged it at 8,000 rpm for 1 minute, and discarded the filtrate.
7. Set the DNeasy column onto a new 2 ml tube, added 500

µl AW1 Buffer, centrifuged it at 8,000 rpm for 1 minute, and discarded the filtrate.

8. Set the DNeasy column onto a new 2 ml tube, added 500 µl AW2 Buffer, centrifuged it at 15,000 rpm for 3 minutes, dried the membrane of DNeasy and discarded the filtrate.
9. Set the DNeasy column onto a new 2 ml tube, added 200 ml AE Buffer consisting of 10 mM Tris-HCl, pH 9.0, 0.5 mM EDTA, kept the solution at room temperature for 1 minute, centrifuged it at 8,000 rpm for 1 minute and eluted the solution.
10. Repeated the step 9.

3. Preparation of primers

The primers requested by NRKK were prepared by Sawady Technology. After the synthesis, they were purified through an Oligopack column (OPC).

Primers for Analysis 1:

Forward: A697f	5'- TACGAAGCCC GTTGGATGAC -3'
Reverse: A1000r	5'- TCGGGTTGAT CGACTGCAGA -3'

Primers for Analysis 2:

Forward: A"479f	5'- TATTCGACGT CGATCGCGGT -3'
Reverse: A"780r	5'- AACTGCTGAG GTGCCGTAGT -3'

4. PCR of the target region

Two PCRs were performed using the two pairs of primers synthesized according to the above method 3, respectively to amplify the targeted regions.

Let's Go PCR	48.5 µL
Primers	0.5 µL (20 µM)
DNA	1 µL
	50 µL

90°C 1 min.
↓ 35 cycles of the following 3 steps were repeated
95°C 30 sec.
50°C 30 sec.
68°C 30 sec.
↓
72°C 30 min.
4°C for stock

After confirming the amplified regions by electrophoresis, the desired regions were cut out and purified using a High Pure PCR Product Purification Kit (Roche Diagnostics).

5. Determination of the nucleotide sequences

The PCR products amplified in the above method 4 were analyzed by an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Japan) in accordance with the protocol supplied by the manufacturer as explained briefly below.

Terminator Ready Reaction Mix	8.0 μL
Template	50 ng
Primer	4 pmol
dH2O	balance to 20 μL
Total	20 μL

PCR condition is as follows:

25 cycles of the following steps

96°C	10 sec
50°C	5 sec
60°C	4 min

3 μL of each sample was applied to an ABI Prism 377 apparatus. The electrophoresis was run overnight.

Results:

1. Preparation of chromosomal DNA

The DNA prepared using DNeasy was dissolved in 100 μL of AE Buffer consisting of 10 mM Tris-HCl, pH 9.0, 0.5 mM EDTA, and the DNA prepared by using SepaGene was dissolved in 50 μL of TE Buffer consisting of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, which were measured for the concentrations.

[Data will be pasted.]

2. PCR

Analysis 1: designated as Enzyme A

304 bp

Analysis 2: designated as Enzyme A"

302 bp

The electrophoresis pattern revealed that the PCR products having the target sizes were obtained.

2% agarose gel and each 3 μL of the PCR products were used for the electrophoresis.

[Picture of electrophoresis gel will be pasted.]

3. Determination of the nucleotide sequences

In accordance with the request, the respective samples were used for bidirectional sequencing (from both the direction) using forward and reverse primers.

The sequences determined are as described in Attachment (B):

The chromatograms are also attached hereto as Attachment (C).

We the undersigned each affirm that this Experimental Report is a true and accurate description of the cloning and sequencing of the amplified products set forth in Attachment B, which products were isolated from the chromosomal DNA of *Gluconobacter oxydans* DSM 4025 supplied to us by K.K. Kyurin

Dated:_____ For: K.K. Sawady Technology Co. Ltd.

By: _____

Masao Mashita, Sales & Marketing Director

Dated: _____ By: _____

日付 Masanori Masuo, COO

Attachment (A)

Reagents

- NS2 medium (supplied by the Requester)
- Reagent for extracting nucleic acids, SepaGene (Sankou Junyaku K.K)
- DNeasy Tissue Kit (K.K. Qiagen)
- ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Japan)
- AmpliTaq DNA Polymerase (Applied Biosystems Japan)
- Let's Go PCR Kit (K.K. Sawady Technology)
- High Pure PCR Purification Kit (Roche Diagnostics K.K.)

Instruments

- Sanyo Incubator MIR153 (Sanyo)
- ABI Prism 377 (Applied Biosystems Japan)
- GeneAmp PCR System 9600 (Applied Biosystems Japan)
- Minicycler (MJ Research)
- MicroCen 13D (Herolab)
- Mupid mini-gel electrophoresis apparatus (Mupid)

Attachment (B)

Sequences of the amplified products.

39F903 (697-1000)/A697f.Seq

TTNCGTGCCT GGGGCCAGAT CACCTATGAC CCCGTCACCA ACCTTGTCCA
CTACGGCTCG ACCGCTGTGG GTCCGGCGTC GGAAACCCAA CGCGGCACCC
CGGGCGGCAC GCTGTACGGC ACGAACACCC GTTTCGCCGT GCGTCCTGAC
ACGGGCGAGA TTGTCTGGCG TCACCAGACC CTGCCCCGCG ACAACTGGGA
CCAGGAATGC ACGTTCGAGA TGATGGTCAC CAATGTGGAT GTCCAACCCT
CGACCGAGAT GGAAGGTCTG CAGTCGATCA ANCGAAANNN NNNNNNNNNN
NNNNN

41F903 (697-1000)/A1000r.Seq

TTCCTCTTGG TCGAGGGTTG GACATCCACA TTGGTGACCA TCATCTCGAA
CGTGCATTCC TGGTCCCAGT TGTCGCGGGG CAGGGTCTGG TGACGCCAGA
CAATCTCGCC CGTGTCAGGA CGCACGGCGA AACGGGTGTT CGTGCCGTAC
AGCGTGCCGC CCGGGGTGCC GCGTTGGGTT TCCGACGCCG GACCCACAGC
GGTCGAGCCG TAGTGGACAA GGTTGGTGAC GGGGTCATAG GTGATCTGGC
CCCAGGCACC GGTCATCCAA CGGGCTTTGT AANNNNNNNN NNNNNNNNNN
N

43F903 (479-780)/A479f.Seq

AAAGCACTTT ATGGNCTCGA ACTCTCCGGC CCGATTGTCG CCAATGGCGT
CATCGTTGCG GGCTCGACCT GTCAGTATTC GCCGTTCGGC TGTTTCGTTT
CGGGCCACGA CTCGGCCACC GGTGAAGAGC TGTGGCGCAA CACCTTTATC
CCGCGCGCCG GCGAAGAGGG TGATGAGACC TGGGGCAATG ATTACGAGGC
CCGCTGGATG ACCGGCGTTT GGGGCCAGAT CACCTATGAC CCCGTTGGCG
GCCTTGTCCA CTACGGCACC TCAAGAGTTA ANANNNNNNN NNNNNNNNN

45F903 (479-780)/A780r.Seq

GACAAGGCTN NCACGGNGTC ATAGGTGATN TGGCCCCAAA CGCCGGTCAT
CCAGCGGGCC TCGTAATCAT TGCCCCAGGT CTCATCACCC TCTTCGCCGG
CGCGCGGGAT AAAGGTGTTG CGCCACAGCT CTTCACCGGT GGCCGAGTCG
TGGCCCGAAA CGAAACAGCC GAACGGCGAA TACTGACAGG TCGAGCCCGC
AACGATGACG CCATTGGCGA CAATCGGGCC GGACGAGTTC GAGACCATAT
CCGTGCCTTG ACCGCGATCG ACGTCCATAA ANNNNNNNNN NNNNNNNNNN

Attachment (C)

[Chromatograms to be pasted.]

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Roche Vitamins AG
Grenzacherstr. 124
CH-4070 Basel

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Roche Vitamins AG Address: Grenzacherstr. 124 CH-4070 Basel	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 14798 Date of the deposit or the transfer[1]: 2002-02-01

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2002-02-01 [2].
On that date, the said microorganism was

( )[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss Date: 2002-02-08

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 12/2001

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Roche Vitamins AG
Grenzacherstr. 124
CH-4070 Basel

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: HB101 pSSA102R	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 14798

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(x) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2002-02-01 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit)
and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiks
Address: Mascheroder Weg 1b, D-38124 Braunschweig	Date: 2002-02-08

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001